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ALCOHOL CONSUMPTION AND BREAST CANCER RISK – MODIFICATION BY GENETIC SUSCEPTIBILITY

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Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the MSc degree in Epidemiology

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Abstract

Gene-environment and gene-gene interactions lie at the root of many human diseases. This thesis evaluated the risk of bias in the case-only design applied to studies of genetic interaction by way of a systematic review and meta-regression analysis. The case-only design was then used to investigate interactions between *BRCA* gene mutations and alcohol consumption among breast cancer patients, and the results were compared to a case-control analysis of *BRCA* mutation carriers with and without breast cancer. The systematic review suggests that the case-only design is unbiased when applied to studies of genetic interaction. The case-control analysis found that increasing wine consumption may reduce the risk of breast cancer among *BRCA1* mutation carriers, and the results of case-only analysis may be compatible with this. Among *BRCA2* mutation carriers, consumption of alcohol other than wine increased breast cancer risk in the case-only analysis while no association was observed in the case-control analysis.

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1. Introduction

1.1. Background and Rationale

1.1.1. Breast Cancer Epidemiology

Breast cancer is the most commonly diagnosed cancer among women worldwide.(1) It is more commonly diagnosed in developed regions (67.8 per 100,000 persons per year) than in developing regions (23.8 per 100,000 persons per year),(1) but is increasing in the latter.(2) In Canada, a woman's lifetime risks of developing and dying from breast cancer are 11.1% and 3.6% respectively.(3) This means that approximately 1 in 9 Canadian women will develop breast cancer in her lifetime and 1 in 28 will die from the disease.

Breast tissue is composed of fat, glandular tissue (arranged in lobes), ducts, and connective tissue, with the relative composition depending on life stage.(2) Breast cancers are most often derived from the epithelial cells lining the ducts (2) and arise as a result of genetic mutations in these cells and proliferation of these cells.(1) Hormones in particular are associated with this process. Estrogen induces breast cell proliferation, allowing either random genetic errors or errors resulting from exogenous DNA insults to accumulate.(4) Accordingly, earlier age at menarche, later age at menopause, low parity, later age at first birth, and shorter duration of breastfeeding, all of which increase lifetime exposure to estrogen, have been associated with an increased breast cancer risk.(5)

Exogenous factors also impact breast cancer risk. Use of hormonal contraceptives transiently increases breast cancer risk for approximately ten years after stopping use.(6) Likewise, current use of hormone replacement therapy (HRT), or use within the last five

years, slightly increases the risk of breast cancer.(7) Body fatness reduces breast cancer risk among premenopausal women by increasing the number of anovulatory menstrual cycles.(2) In postmenopausal women, body fatness increases breast cancer risk, likely by increasing circulating insulin, insulin-like growth factors, and estrogens, creating a favourable environment for cancer initiation and progression. A reversal of this process is believed to drive the reduction in risk associated with physical activity among postmenopausal women.(2)

1.1.1.1. Alcohol Consumption and Breast Cancer Risk

The International Agency for Research on Cancer (IARC) classifies ethanol, the active ingredient in alcoholic beverages, as a human carcinogen.(8) A pooled analysis of 6 cohort studies from Canada, the Netherlands, Sweden, and the United States (US) found a risk increase of 1.09 (95% CI, 1.04-1.13) per 10 g/day of alcohol consumed.(9) Similarly, a pooled analysis of 53 case-control studies by the Collaborative Group on Hormonal Factors in Breast Cancer found that the relative risk of breast cancer increased by 7.1% (95% CI 5.5-8.7%) per 10 g/day alcohol consumed.(10) Based on a review of the worldwide epidemiological evidence on breast cancer and alcohol consumption, including the two aforementioned pooled analyses, the 2007 World Cancer Research Fund / American Institute for Cancer Research (WCRF / AICR) report (2) concluded that:

> *"There is ample, generally consistent evidence from case-control and cohort studies. A dose-response relationship is apparent. There is robust evidence for mechanisms operating in humans. The evidence that alcoholic*

drinks are a cause of premenopausal and postmenopausal breast cancer is convincing. No threshold was identified."

There are several potential mechanisms by which alcohol consumption may increase breast cancer risk. These include through DNA damage caused by both the metabolism of ethanol in breast tissue, producing reactive oxygen species and the weak carcinogen acetaldehyde,(11) and through the disruption of DNA repair mechanisms by ethanol.(12) Alcohol also interferes with estrogen metabolism and response. Higher levels of circulating estrogens have been associated with alcohol consumption.(13-15) In vitro, ethanol increases cellular responsiveness to estrogen by down-regulating the tumor suppressor gene *BRCA1*, an inhibitor of estrogen receptor alpha (ER-α) activity, and increasing the transcriptional activity of ER- α.(16)

1.1.2. Mutations in the *BRCA1* and *BRCA2* Genes

A hereditary basis for breast cancer was described as early as 1866. However, these multiple-case families did not garner attention until nearly a century later.(17,18) Genetic linkage studies in the 1980s were fundamental to the mapping and sequencing of the two major breast cancer susceptibility genes identified to date: *BRCA1*, sequenced in 1994,(19) and *BRCA2*, sequenced a year later.(20) Of the 10% of breast cancers that are hereditary, 16% can be attributed to a mutation in either of these two genes.(17)

To date, more than 1000 deleterious mutations in *BRCA1* and 500 deleterious mutations in *BRCA2* have been identified.(21) These mutations are estimated to have arisen within the last 100 generations and therefore tend to be population-specific.(17) For example, founder mutations have been identified in Ashkenazi Jewish,(22) Icelandic,(23) Polish, (24) Dutch, (25) and French-Canadian (26) ethnic groups.

Determining the prevalence of mutation carriers in an ethnically diverse population is difficult due to the number of possible mutations. Model-based approaches (27-31) have yielded population estimates for both genes combined of between 0.21% in the United Kingdom population (31) to 1.01% in the Ontario population.(27)

BRCA1 and *BRCA2* are integral to genome stability.(32-34) Both *BRCA1* and *BRCA2* gene products are involved in the repair of double strand breaks (DSB) via homologous recombination (HR).(32) Additionally, BRCA1 is involved in cell cycle signaling, transcription, chromatin remodeling, the breakdown of proteins through a cellular process known as ubiquitination,(33) and control of estrogen-mediated cell proliferation through the regulation of ER- α.(35,36) Inherited mutations in *BRCA1* and *BRCA2* primarily predispose carriers to breast and ovarian cancer, although higher risks of pancreatic and prostate cancer in *BRCA2* carriers have also been found.(21)

Penetrance, the proportion of those with mutations who develop disease, can be calculated from high-risk families using the maximum logarithm of the odds (LOD) score approach, or from population-based series of cases unselected for family history by calculating the incidence of cancer among relatives of mutation carriers, the kin-cohort approach.(17) Estimates derived from the kin-cohort approach are more applicable to the majority of mutation carriers. A meta-analysis of 22 studies that used the kin-cohort approach determined that the average cumulative risk of breast cancer by age 70 years was 65% (95% CI 44%-78%) in *BRCA1* carriers and 45% (31%-56%) in *BRCA2* carriers.(37) The average cumulative risk of ovarian cancer by age 70 years was 39% (18%-54%) in *BRCA1* carriers and 11% (2.4%-19%) in *BRCA2* carriers.

Penetrance is affected by mutation-specific (e.g. type, location of mutation), genetic, and lifestyle factors.(21,27,38) For example, mutations in a 3.3 kb region of *BRCA2*, termed the ovarian cancer cluster region (OCCR), confer a high risk of ovarian cancer but a lower risk of breast cancer.(21) Higher penetrance estimates calculated from family members of index cases diagnosed at less than 35 years of age are consistent with clustering of other genes that modify breast cancer risk in these families.(37) The increase in breast cancer risk among more recent birth cohorts of mutation carriers supports the existence of lifestyle and environmental risk modifiers.(37)

It should not be assumed that breast cancer risk modifiers identified in the general population have the same effect among mutation carriers.(38,39) Based on studies of affected and unaffected mutation carriers, early age at menarche increases breast cancer risk among *BRCA1* mutation carriers but has no effect among *BRCA2* mutation carriers. Increasing parity is associated with an increased risk of breast cancer diagnosed at a younger age, especially among *BRCA2* mutation carriers, whereas the effect of early age at first birth is unclear. Breastfeeding may reduce the risk of breast cancer among mutation carriers. Oral contraceptive use may increase the risk of breast cancer, but results have been inconsistent; little research has been done on the effects of HRT use among mutation carriers. Physical exercise and healthy body weight are associated with a reduced breast cancer risk, but this is based on only two studies. Alcohol consumption appears to have little effect on breast cancer risk, but has also been investigated in only two studies.(40,41) Differences in risk associated with breast cancer risk factors between women in the general population and women with *BRCA* gene mutations are suggestive of interaction.

1.1.3. The Case-Only Study Design

Conventional studies of gene-environment and gene-gene interactions, including case-control studies, are often hampered by power limitations and difficulties finding a suitable control group.(42,43) The case-only study design requires smaller sample sizes to detect interactions than the case-control design (44,45) and avoids problems with control group selection. By assuming independence between genotype and environment in the source population, the odds ratio calculated from cases only is a measure of the departure of the joint effects of genotype and environment from a multiplicative model of interaction.(43,46) The same applies to studies of gene-gene interaction. Despite the advantages of the design, it has been criticized for its potential susceptibility to bias caused by violations of the independence assumption, leading some authors to question its utility.(47,48) However, the extent of this potential bias has not been evaluated in an empirical assessment across studies.

1.2. Objectives and Scope of Thesis

The objectives of this thesis are:

I. To evaluate the utility of the case-only design in studies of gene-gene and gene-environment interaction. This is addressed in Chapter 2 by means of a systematic review of the epidemiological literature in this area and a meta-regression analysis comparing interaction estimates obtained from case-only and case-control analyses of the same dataset.

II. To apply the case-only design to measure interactions between alcohol consumption and mutations in the *BRCA1* and *BRCA2* genes in determining

breast cancer risk. This is addressed in Chapter 3 using a sample of French-Canadian breast cancer patients tested for *BRCA* gene mutations.

III. To determine the risks of breast cancer associated with alcohol consumption among *BRCA1* and *BRCA2* mutation carriers using a case-control design of *BRCA* mutation carriers and to compare the results with the case-only analysis. This is addressed in Chapter 4 using matched pairs of affected and unaffected mutation carriers from 54 centres in 8 countries.

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2. Bias in the case-only design applied to studies of gene-environment and gene-gene interaction; a systematic review and meta-analysis

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2.1. Abstract

BACKGROUND: The case-only study, proposed as a design specifically for assessing departure from multiplicative gene-environment and gene-gene interactions, is of considerable potential value but there are concerns about its validity. The objective of this study was to evaluate the extent and sources of bias in the case-only design by means of a systematic review and meta-regression analysis.

METHODS: The MEDLINE, CINAHL, EMBASE, and PUBMED databases were searched through to October 7, 2009. Studies that assessed bias in the case-only design applied to the study of gene-environment and gene-gene interaction were identified. Qualitative comments on the sources and extent of bias were extracted. A meta-regression analysis of the ratio (IOR_{CC}/IOR_{CO}) of the case-control (IOR_{CC}) and case-only (IOR_{CO}) interaction odds ratios was conducted based on studies in which both methods were applied to the same dataset.

RESULTS: The search yielded 365 unique articles, of which 38 met the final inclusion criteria. Potential sources of bias in the case-only design included non-independence of genotype and exposure in the source population. Meta-regression analysis, based on 24 evaluations, produced a mean of the ratio IOR_{CC}/IOR_{CO} of 1.06 (95% CI, 0.93-1.22), suggesting that bias in case-only designs is not common in practice. The I^2 statistic indicated that 23.9% of the between-study variation was due to true heterogeneity, which was not explained by any methodological characteristics of the included studies.

CONCLUSION: As understanding of the relationships between genes and environmental exposures in the population improves, the case-only design may prove to be of considerable value.

2.2. Introduction

The results of recent genome-wide association studies of common chronic diseases have generated considerable excitement.(1,2) However, the strength of the association between genes and diseases observed in these studies has been weak, and a large proportion of familial clustering of disease remains unexplained.(3) A combination of genes, or a combination of genes with one or more environmental exposures, may account for some of this 'missing' heritability. The investigation of the gene-environment interactions thought to be of key importance in the etiology of cancer and other chronic diseases will be the subject of intense investigation through the harmonization of existing and planned large scale cohort studies.(4,5) Nonetheless, it is likely to be extremely difficult even for the largest cohorts to amass sufficient cases to enable the investigation of gene-environment or gene-gene interactions,(6) especially in relation to the less common cancers.(7)

The case-only design has received considerable attention with respect to its potential use in the investigation of gene-environment and gene-gene interactions.(8-10) The design may be especially useful for rare diseases because the sample sizes required to detect interaction between genes and environmental exposures, or simply between two genes, are smaller for the case-only design than, among others, the case-control design.(11,12) The case-only approach would also potentially be valuable when it is difficult to recruit control subjects, or when, in a multicentre study, controls are not comparable among centres because of different methods of recruitment and/or exposure assessment.

In the investigation of gene-environment interaction, the validity of the case-only approach depends on the independence of genotype and exposure in the source

population.(13,14) Similarly, investigation of gene-gene interaction requires that the genotypes are independent in the source population.(15) (For a detailed description of the derivation of the case-only estimate of interaction and its relationship to the cohort and case-control estimates of interaction, see Appendix A.) The principles involved in the study of gene-gene interaction are identical to those involved in gene-environment interaction. Therefore, to avoid repetition, we have written only about gene-environment interaction, but the findings apply equally to gene-gene interaction. To our knowledge, no systematic evaluation of the strengths and limitations of the case-only design has been carried out. The present study aims to determine the sources and extent of bias arising from the application of the case-only design in studies of gene-environment and gene-gene interaction by way of a systematic review of the epidemiological literature in this area and a meta-regression analysis comparing interaction estimates obtained from case-only and other analyses of the same datasets.

2.3. Methods

The conduct and reporting of the systematic review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement guidelines.(16)

2.3.1. Search Strategy

Articles were identified through the use of MeSH terms and text word searches for 'genetics' and 'case-only' (Appendix 2). The search was executed in MEDLINE, CINAHL, EMBASE, and PUBMED and included articles published through to October 7, 2009. The clinicaltrials.gov site was also searched up to November 9, 2009 using text words. Reference lists of chapters on the case-only design in genetic epidemiology textbooks(17-19) were hand

searched. Searches were restricted to English language studies published after 1994, the year the case-only design was first proposed.(8)

2.3.2. Inclusion and Exclusion Criteria

Study inclusion criteria were: (i) a case-only study design or a discussion of the case-only design; (ii) assessment of at least one genetic risk factor for the outcome of interest; and (iii) an evaluation of bias associated with the application of the case-only design in studies of gene-environment or gene-gene interaction. Bias was defined as a distortion of the measure of association. Studies that looked at type I and II errors were not considered. Studies that compared the case-only design with variations of the case-control design (e.g. studies that assessed genotype or exposure among only a subset of controls) were not considered. Studies that looked at interactions with somatic gene mutations were also not included, as this type of interaction is interpreted differently from interactions with germline genetic variation,(20) for which the case-only approach was specifically designed.(8)

2.3.3. Study Selection

Titles and abstracts of identified studies were screened, with those meeting the first two inclusion criteria proceeding to the full text screen. At this stage, studies without an assessment of bias were excluded. Screening was completed by two reviewers (JD and MG or JF) with disagreements resolved by consensus.

2.3.4. Qualitative Synthesis

Qualitative comments on the sources and extent of bias associated with the case-only design were extracted in duplicate (JD, MG) from included studies. Comments that were cited from another publication were not eligible.

2.3.5. Quantitative Synthesis

Case-only and cohort interaction estimates are theoretically equivalent when genotype and environment are independent in the source population, and case-only and case-control interaction estimates are equivalent when genotype and environment are independent among controls (Appendix A). We therefore considered for inclusion in the meta-regression analysis studies that compared estimates of interaction derived from a case-only analysis to those derived from a cohort or case-control analysis of the same dataset. Furthermore, to be included, the data could not have been simulated, the case-only interaction odds ratio (IOR_{CO}) had to have been reported in the text or in tables, including web tables, as did the corresponding cohort interaction relative risk (IRR_{true}) or case-control interaction odds ratio (IOR_{CC}) (or a 2x4 table from which the IRR_{true} or IOR_{CC} could be calculated). The same cases had to have been used in both study designs and genotype and environment had to have been measured in the same manner.

Ultimately, all of the eligible empirical evaluations compared a case-only design to a case-control design. The IOR_{CC} and IOR_{CO} were calculated manually from 2x4 tables that were presented in the published article or that were requested from study authors. The ratio of the case-control odds ratio to the case-only odds ratio (IOR_{CC}/IOR_{CO}) was calculated for each interaction. The variance of this ratio is the variance of the gene-environment or gene-gene association calculated among controls in the 2x4 table, since this should be the only difference between the IOR_{CC} and IOR_{CO} (see equation (2), Appendix A). If 2x4 tables were not available, the IOR_{CC} and IOR_{CO} presented in the published article were used to calculate the IOR_{CC}/IOR_{CO} ratio and the variance was imputed based on the arithmetic mean variance from the other studies included in the meta-regression.

Information was extracted from each study on the control group selection (source and selection of controls, matching), testing of the independence assumption, testing of Hardy-Weinberg equilibrium (HWE), participation rate, number of interactions tested, type of interaction (gene-environment or gene-gene), and whether the data were from a primary study or a methodological study that used data from a secondary source. If this information was not reported (for example in studies using data from a secondary source) it was sought from the original publication.

If multiple interactions from independent studies were presented in an article, each was eligible for the meta-regression analysis. If multiple interactions were presented from a single study, the largest IOR_{CC}/IOR_{CO} ratio was used in the meta-regression analysis. If 2x4 counts were available for some but not all interactions in a study, the interaction with the largest ratio was selected from this subset of all interactions. Interactions were not eligible if genotype and environment were not independent in controls or if it was known that two genes were linked (i.e. not independent) in a study of gene-gene interaction. Study selection and data extraction for the meta-regression analysis were completed by one reviewer (JD) and uncertainties were resolved by consultation with co-authors.

2.3.6. Statistical Methods

All analyses were done with Comprehensive Meta Analysis V2. A random effects model was fit to the data and heterogeneity was assessed by the Q-statistic and I^2. Influence of study design parameters on the mean IOR_{CC}/IOR_{CO} ratio was explored in subgroup analyses for categorical parameters and by univariable meta-regression for continuous parameters. The strength of the relationship between the study design parameters and the mean ratio was

quantified by the Q-test based on analysis of variance. The proportion of variance explained by each design parameter was assessed by R^2.

2.4. Results

The database and textbook searches yielded 365 unique articles, of which 38 met the final inclusion criteria (Figure 2-1). The types of studies identified are summarized in Table 2-1.

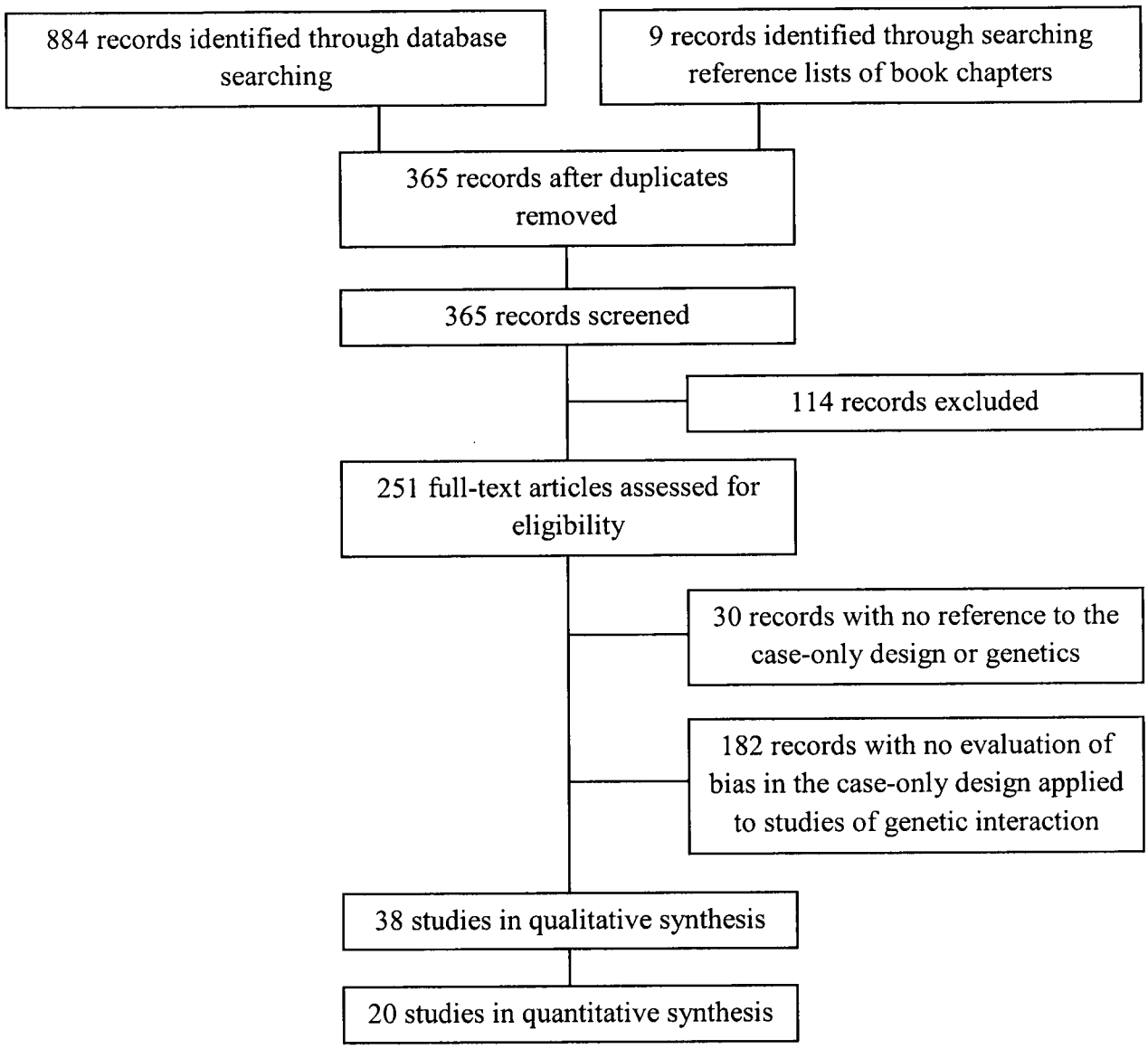

Figure 2-1. Flow of information through different phases of the systematic review.

Table 2-1. Types of studies included in the systematic review[a].

Study Type	Number of Studies	Citation(s)
Theoretical evaluation	10	(8,14,22,25,26,30,32,33,35,55)
Theoretical evaluation with an empirical comparison of case-only and case-control designs using real data	9	(9,13,15,24,27,28,31,39,58)[b]
Empirical comparison of case-only and case-control designs using real data	12	(36,44,59-68)
Empirical comparison of case-only and case-control designs using simulated data	1	(40)
Empirical comparison of case-only meta-analysis and case-control meta-analysis using real data	3	(37,38,69)[c]
Empirical comparison of pooled case-only and pooled case-control designs using real data	1	(70)
Empirical comparison of case-only and another study design using real data	2	(42,71)

[a]Theoretical evaluations were those that assessed bias in the case-only design using mathematical arguments or simulations. Empirical evaluations were those that compared estimates of interaction obtained from case-only and other analyses of the same datasets.
[b](13) also had an empirical comparison of case-only and cohort designs using simulated data.
[c](38) also had an empirical comparison of case-only and case-control designs using real data; (37) compared a case-control meta-analysis to a case-only analysis

2.4.1. Qualitative Comments

Extracted qualitative comments are presented in Appendix C. Five studies evaluated the sensitivity of the case-only design to bias caused by non-independence of genetic and environmental factors in the source population.(21-25) All showed that bias could be substantial under non-independence. Many studies discussed potential sources of non-independence, including known or symptomatic gene status that may alter behavior, population stratification, and linkage disequilibrium.(8,9,15,21-23,26-28) Wang(25) showed that population stratification bias would be negligible if exposure prevalence odds and genotype frequency odds were

uncorrelated across the strata, and if there were no variation in either the exposure prevalence odds or genotype frequency odds.

Since independence of genotype and exposure in the source population is needed to ensure that a case-only study produces a valid estimate of the true interaction effect from a cohort study (Appendix A), some authors have considered how this assumption can be evaluated. When genotype and exposure data exist for cases and controls, it may be possible to verify the independence of genetic and environmental factors among controls before proceeding to the more statistically efficient case-only analysis.(21,26,29) However, Gatto et al (23) discussed how the gene-environment association in controls will only be an accurate reflection of that in the source population if "...the baseline risk of disease is small (<1%) and the interaction and independent effects are moderate ([i.e. risk ratio] <2). Alternatively, controls can be used if the disease risk is low (e.g. <5%) in all strata of genotype and exposure." When these conditions do not apply, Gatto et al have shown that "using controls to approximate the gene-environment odds ratio in the underlying population can lead to the rejection of valid case-only data, an overestimation of the underlying interaction, or a finding of interaction when none exists."

Five studies found that non-independence of genetic and environmental factors in the control population would bias the case-only estimate of interaction relative to the case-control estimate of interaction. (21,23,28,30,31) Schmidt and Schaid (26) showed that the case-only estimate of interaction will under-estimate the case-control estimate of interaction when controls are selected from survivors at the end of the study time (cumulative sampling) or longitudinally throughout the course of the study (density sampling) unless "the disease is truly rare (on the order of 1%) or if the disease is somewhat more common (around 5%) but the genes under study confer only moderately increased disease risk (e.g. OR_g<6)." This occurs for two reasons.

First, the odds ratio calculated from a case-only analysis under the independence assumption estimates the population interaction risk ratio (see equation (1), Appendix A) whereas the odds ratio calculated from a case-control study estimates either the population interaction risk ratio, odds ratio, or rate ratio, depending on how controls are selected. If controls are sampled from the base population at the beginning of a study, the case-control odds ratio estimates the population relative risk. However, if controls are selected by cumulative or density sampling, the case-control odds ratio estimates the population interaction odds ratio or interaction rate ratio, respectively. These statistics approximate the interaction relative risk if the disease is rare.

Second, if in the source population during the reference period for exposure, a combination of exposure and genotype affects survival, then selection of controls from survivors at the end of the study or longitudinally throughout the study will result in non-independence of genotype and exposure among controls. If such a case-control study were compared to a case-only analysis, the results would differ.(32) This difference would not occur if controls were selected from the base population at the beginning of a study.

Two studies looked at the effects of misclassification in case-only studies. Cheng(33) showed that random genotyping error would bias case-only estimates of gene-environment interaction relative to case-only estimates obtained under no genotyping error. He proposed genotyping at least some study subjects twice and incorporating the information on error rate in the analysis to adjust for genotyping error. He also noted that when there truly was no interaction effect, the case-only estimate would have a value of unity, indicating no interaction, even in the presence of genotyping error. This has also been observed for the case-control estimate of interaction.(34) In a subsequent paper, Cheng and Lin(35) showed that as long as

both the true and misclassified genotype and exposure were conditionally independent given a third stratification variable (such as ethnicity), the joint effect of genotype and exposure misclassification in the case-only study would be negligible in a model that controlled for this stratification variable.

2.4.2. Meta-Regression Analysis

Six comparisons of the case-only and case-control designs using real data were excluded from the meta-regression analysis because of differences in the case population used in both study designs,(36-38) because neither the IOR_{CC} nor 2x4 tables was presented,(27,39) or because no IORs were presented.(28) Other excluded empirical evaluations included one comparison of the case-only and case-control designs using simulated data,(40) one comparison of the case-only and cohort designs using simulated data and in which no IRR_{true} was reported,(13) one comparison of case-only and kin-cohort designs,(41) and one comparison of the case-only, Cox regression, and case-cohort analysis of clinical trials with failure time endpoints.(42) All evaluations concluded that both methods yielded similar results, with the exception of two evaluations. Albert et al showed that the case-only estimate would be biased when genotype and exposure were not independent, whereas the cohort design would give valid estimates of interaction.(13) Liu et al found that "five of the seven significant interactions detected in the complete case-control sets were not detected in a case-only analysis... This was due to correlations between G and E [among controls] that were in the opposite direction of the risk interaction effect. Although these correlations were not statistically significant, they diluted the ability to detect interaction in the case-only design."(28) This is an example of the sensitivity of the case-only design to even slight gene-environment associations among controls.

Twenty-five evaluations presented in 20 studies were eligible for the meta-regression analysis (Table 2-2). These included two studies which reported on the same study population;(43,44)so the one that reported 2x4 table counts was used in the analysis(44). A primary study was the source of data in 63% of evaluations (Table 2-3) and most comparisons (83%) were for gene-environment interactions. The number of interactions tested by both study designs ranged from 1 to 88 and the independence assumption was tested among controls in 63% of studies. Controls were equally likely to have been sourced from the general population (46%) as from a hospital or clinic (46%), were more likely to have been selected by density sampling (67%) than cumulative sampling (29%) and were more likely to be unmatched (50%) than matched (42%). Genotypes were found to be in HWE among controls and among unspecified groups in 29% and 21% of evaluations respectively. Sixty-three percent of evaluations reported participation rates.

The variance of the IOR_{CC}/IOR_{CO} ratio was calculated in 22 evaluations and was imputed in 2 evaluations. The mean IOR_{CC}/IOR_{CO} ratio under the random effect model was 1.06 (95% CI, 0.93-1.22) (Figure 2-2). The Q-test for heterogeneity was not statistically significant ($p=0.14$) and the I^2 statistic indicated that 23.9% of the observed between study variation was due to true heterogeneity. Examination of the design features which might have accounted for differences between studies revealed that the number of interactions tested (measured on a continuous scale) accounted for 56.5% of this variation (Table 2-3 and Appendix D). However, after removal of two studies which evaluated more than ten interactions (88 and 32 respectively), the I^2 was reduced to 13.9 and the number of interactions tested was no longer significant ($p=0.36$), nor did it explain any heterogeneity ($R^2=0\%$). Testing for HWE explained 4.3% of the between-study heterogeneity, although differences in the IOR_{CC}/IOR_{CO} ratio between groups

were not significant (p=0.13). None of the other methodological features was substantially or significantly associated with the mean IOR_{CC}/IOR_{CO} ratio. Removal of one study at a time did not change the results (Appendix E), nor did restricting the analysis to the 22 studies for which variance was calculated empirically (IOR_{CC}/IOR_{CO}=1.06, 95% CI 0.92-1.23). Because the number of included studies was small and none of the methodological features was significant in univariable analysis, multivariable meta-regression analysis was not undertaken.

Table 2-2. Characteristics of studies included in the meta-regression analysis.

Citation	Country of Study Site	Number of Interactions Tested and Reported in Both Study Designs	Disease	Gene-Environment Independence	Source of Controls	Matched or Unmatched Controls	Control Sampling	HWE tested	Participation rate[a] (cases)	Participation rate[a] (controls)
Albert, 2001	China	1	Lung cancer	Tested in controls	Cohort of miners (population)	Matched	Density	Not stated	~50% (cohort)	~50% (cohort)
Cáceres, 2005	Chile	9	Prostate cancer	Assumed	Population	Unmatched	Density	Tested – results not stated	Not stated	Not stated
Cáceres, 2009	Chile	3	Lung cancer	Tested in controls	Hospital	Unmatched	Density	Tested - group not specified – in equilibrium	Not stated	Not stated
Chang-Claude, 2003	Germany	1	Breast cancer	Tested in controls	Population	Matched	Density	Tested in controls – in equilibrium	67%	50%
Clavel, 2005	France	32	Leukemia	Tested in controls	Hospital	Unmatched	Density	Tested in controls – in equilibrium	78%	36%
Deng, 2004	Australia	4	Parkinson's disease	Tested in controls	Spouses, Community	Unmatched	Cumulative	Tested in controls – in equilibrium	Not stated	Not stated
Dowling, 2003	USA	3	Venous thromboembolism	Tested in 95% of controls	Clinic	Unmatched	Density	Tested – results not stated	71%	Not stated (pooled 2 groups)
Egan, 2003	USA	4	Breast cancer	Tested in controls	Population	Unmatched	Density	Not stated	60%	51%
Garcia-	Spain	2	Bladder cancer	Assumed	Hospital	Matched	Density	Tested in	82%	81%

Closas, 2005								controls – in equilibrium		
Hamajima, 1999 (Hildesheim, 1997)	Taiwan	1	Nasopharyngeal cancer	Tested in controls	Population	Matched	Density	Tested - group not specified – in equilibrium	96%	86%
Hamajima, 1999 (Wu, 1997)	USA	1	Lung cancer	Tested in controls	Population	Matched	Density	Tested - group not specified – in equilibrium	Not stated	Not stated
Hamajima, 1999 (Taylor, 1998)	USA	1	Bladder cancer	Tested in controls	Clinic	Matched	Density	Not stated	Not stated	Not stated
Hamajima, 1999 (Sugimura, 1998)	Japan	1	Lung cancer	Tested in controls	Hospital	Unmatched	Unclear	Tested - group not specified – in equilibrium	100%	Not stated
Khoury, 1996	USA	1	Cleft palate	Assumed	Birth defect registry (population)	Unmatched	Cumulative	Not stated	75%	75%
Lazo-Langner, 2006	Canada	1	Thrombotic occlusion	Assumed	Dialysis patients (hospital)	Unmatched	Cumulative	Tested in controls – in equilibrium	87% (cohort of dialysis patients)	87% (cohort of dialysis patients)
Li, 2009	China	2	Smoking Initiation	Known (unlinked genes)	Population	Not stated	Cumulative	Tested - group not specified – in equilibrium	97%	97%
Mukherjee, 2008	Israel	4	Colorectal cancer	Tested in controls	Population	Matched	Density	Not stated	Not stated	Not stated
Stücker, 2001 (London,	USA	2	Lung cancer	Tested in controls	Population	Unmatched	Cumulative	Not stated	70%	50%

1995)										
Stücker, 2001 (Stücker, 1999)	France	1	Lung cancer	Tested in controls	Hospital	Matched	Density	Not stated	100%	'A small number refused'
Stücker, 2001 (Jourenkova, 1997)	France	1	Lung cancer	Tested in controls	Hospital	Matched	Density	Not stated	Not stated	Not stated
Theodoratou, 2008	Scotland	88	Colorectal cancer	Assumed	Population	Unmatched	Density	Tested in controls – in equilibrium	58%	57%
Yang, 1999	Ireland	1	Neural tube defects	Not stated	Hospital	Unmatched	Cumulative	Not stated	Not stated	Not stated
Yang, 2005	Japan	6	Esophageal cancer	Not stated	Hospital	Matched	Density	Tested in controls – in equilibrium	Not stated	60%
Zeiger, 2005	USA, Denmark	2	Oral Clefts	Not stated	Population, Clinic	Not stated	Cumulative	Not stated	Not stated	Not stated

[a]Participation rate defined as the number of participants that provided a sample for genotyping over the number of eligible participants. The rate does not include genotyping success rate (if reported separately).
HWE=Hardy-Weinberg equilibrium

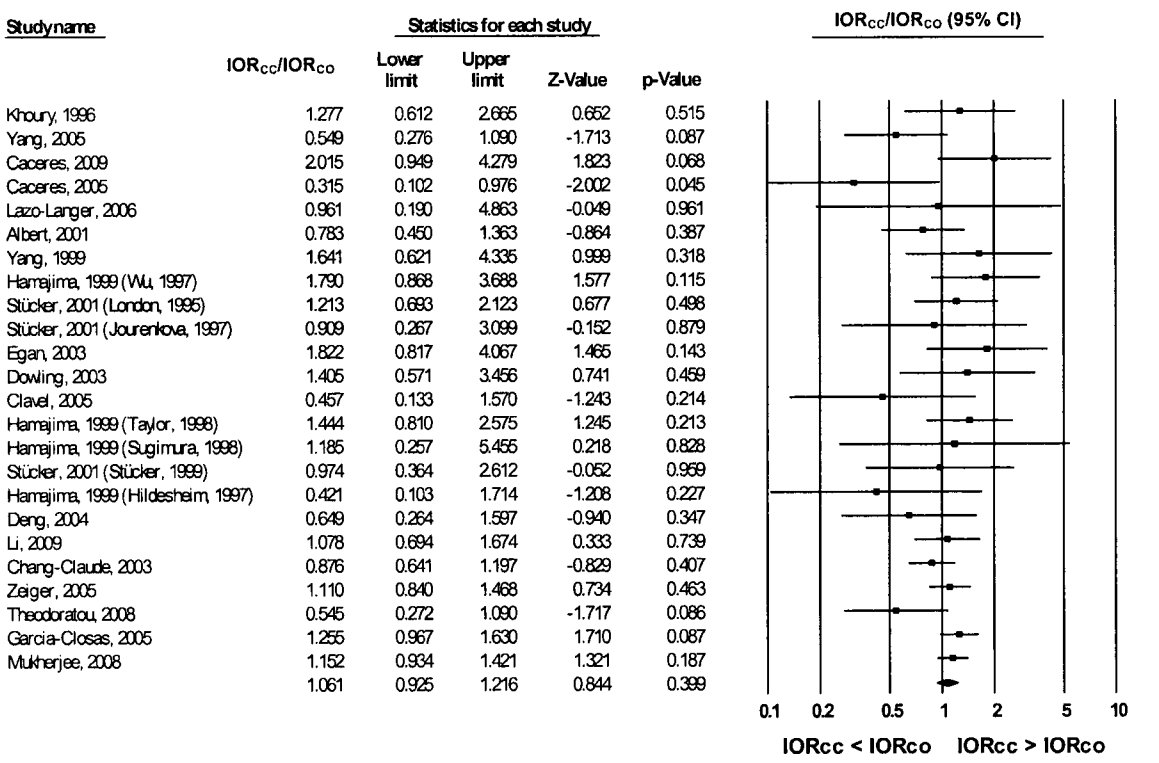

Figure 2-2. Forrest plot of the mean IOR_{CC}/IOR_{CO} ratio calculated from studies included in the meta-regression analysis under a random effect model. Studies are arranged from smallest to largest on the basis of number of cases.

Table 2-3. Univariable meta-regression analysis of study design parameters on the IOR_{CC}/IOR_{CO} ratio.

Methodological Feature	Number of studies (%)	IOR_{CC}/IOR_{CO} Ratio (95% CI)	p-value for heterogeneity (Q statistic)	Proportion of variance explained (R^2)
Study Type				
Methodological	9 (38%)	1.16 (0.92-1.43)	0.32	0%
Primary study	15 (63%)	1.00 (0.83-1.20)		
Interaction				
Gene-environment	20 (83%)	1.04 (0.90-1.21)	0.51	0%
Gene-gene	4 (17%)	1.21 (0.80-1.83)		
Independence				
Assumed	8 (33%)	0.98 (0.76-1.25)	0.75	0%
Tested	15 (63%)	1.10 (0.91-1.35)		
Known	1 (4.0%)	1.08 (0.61-1.92)		
Source of Controls				
Population	11 (46%)	1.01 (0.82-1.23)	0.70	0%
Hospital or clinic	11 (46%)	1.16 (0.90-1.50)		
Other	2 (8%)	1.01 (0.67-1.52)		
Selection of Controls				
Cumulative	7 (29%)	1.12 (0.86-1.45)	0.88	0%
Density	16 (67%)	1.03 (0.86-1.23)		
Unknown	1 (4%)	1.19 (0.25-5.68)		
Cases and Controls Matched				
No	12 (50%)	1.06 (0.80-1.39)	0.97	0%
Yes	10 (42%)	1.04 (0.85-1.28)		
Not stated	2 (8%)	1.10 (0.77-1.57)		
Hardy-Weinberg Equilibrium				
Not stated / Tested - results not stated	12 (50%)	1.13 (0.94-1.37)	0.13	4.3%
Tested – group not specified - in equilibrium	5 (21%)	1.29 (0.90-1.85)		
Tested in controls - in equilibrium	7 (29%)	0.87 (0.69-1.11)		
Participation Rate				
Not reported	9 (38%)	1.18 (0.95-1.46)	0.20	0%
Reported	15 (63%)	0.98 (0.82-1.18)		
Number of Interactions				

1	13 (54%)	1.07 (0.88-1.30)	0.10	0%
2 or 3	4 (17%)	1.34 (1.00-1.80)		
4 or more	7 (29%)	0.87 (0.67-1.13)		
Number of Interactions (continuous)				
Slope	24 (100%)	-0.01 (-0.02-0.00)	0.03	56.5%

2.5. Discussion

Many papers included in this systematic review emphasized that genetic and environmental factors must be independent in the source population for the case-only design to provide an unbiased estimate of the true interaction effect. They explained why independence could not be assumed in all circumstances, and why testing the independence assumption in disease-free controls could be problematic if the disease were not rare. Several papers discussed how case-only and case-control odds ratios would be discrepant if genetic and environmental factors were not independent among controls, and that this would be more likely to occur if controls were selected using density or cumulative sampling and either the disease was not rare, or the study was of a survival trait. Two studies demonstrated that misclassification of genetic and/or environmental factors could bias the case-only odds ratio and suggested analytic techniques to adjust for this.

In the meta-analysis, no substantial or significant difference between the case-only and case-control interaction odds ratios was found. There was some between-study heterogeneity in the IOR_{CC}/IOR_{CO} ratio, but this was largely explained by two studies that evaluated many interactions and were therefore more likely to find a chance difference in the IOR_{CC} and IOR_{CO} estimates. Nonetheless, the findings from the meta-analysis do not imply that either the case-

only or case-control odds ratio is unbiased relative to the true interaction risk ratio. From Appendix A, IOR_{CC} will approximate IRR_{true} if the disease is rare or if genetic and environmental factors are independent in both the source population and in controls, whereas IOR_{CO} will approximate IRR_{true} if genetic and environmental factors are independent in the source population, regardless of whether the rare disease assumption holds.

Independence of genes and exposures at the population level is key to ensuring valid estimates from the case-only design, yet it has rarely been empirically tested. Theoretically, Mendelian randomization, the principle that alleles will segregate randomly in the population, regardless of environmental exposures,(45,46) supports the likelihood of independence.(14) Indeed, Davey-Smith et al found no significant associations of 23 genetic variants with either each other, or with 96 behavioural, socioeconomic, and physiological factors among 4,286 women in the British Women's Heart and Health Study.(47) Further evidence will come from biobanks such as those contributing to the Public Population Project in Genomics (www.p3g.org), and from large cohort studies such as the US National Health and Nutrition Examination Study III (NHANES III) Collaborative Genomics Project(5) and the Canadian Health Measures Survey.(48)

Of the studies included in the meta-analysis, none assessed independence in the source population, though several assessed independence among controls. Provided that tests of gene-environment association among controls were valid estimates of the association in the source population, then the studies included in the systematic review generally supported the likelihood of independence because studies that assumed independence, compared to those that evaluated independence among controls, had similar IOR_{CC}/IOR_{CO} ratios. Associations between genotype

and exposure were, however, found among controls in four studies (21,24,31,43) although two of these studies (21,24) had expressly selected these associations from secondary data sources to prove that the case-only study was biased under these circumstances. Because the assumption of independence was violated, these associations were not eligible for the meta-regression analysis.

In all of the studies included in the meta-analysis, controls were selected either from survivors at the end of the study time (cumulative sampling) or longitudinally throughout the study time (density sampling). Thus, non-independence of genetic and environmental factors among controls was theoretically plausible.(26) However, none of the genes under study is known to confer a significantly increased risk of disease or to be associated with longevity. Therefore, any association between genetic and environmental factors in the control group as a result of control group selection is expected to be minimal. This is further affirmed by the scarcity of significant gene-environment associations found among tested controls.

Although differential misclassification by case-control status is not a concern in case-only studies, misclassification of genetic and environmental factors can, except under certain circumstances, seriously distort interaction estimates,(33) as it can in case-control studies.(34) The likelihood of genetic misclassification is affected by the rate of genotyping error, which can reportedly range from 0.5% to 30% (49) depending on factors related to the DNA sequence itself, low quality or quantity of DNA, biochemical artefacts and equipment errors, and human errors in sample and data handling.(50) Lack of HWE among controls may be an indication of genotyping errors or peculiarities in the dataset,(17,51,52) although power to detect deviations from HWE among controls may be limited.(53) For example, a simulation study failed to detect deviations from HWE when errors were introduced into the dataset.(54) Given this uncertainty,

as well as documented under-reporting of violations of HWE and confusion over which groups should be tested for HWE,(53) the STREGA (Strengthening the Reporting of Genetic Association Studies) statement recommended that authors clearly state whether HWE was evaluated, and, if so, how it was tested, so that empirical evidence may accrue.(49) To this end, testing for HWE was included as an item in the meta-regression analysis. It was found that studies that did and did not report testing for HWE, regardless of the group in which HWE was tested, reported similar IOR_{CC}/IOR_{CO} ratios. However, it is unclear if this was because HWE was not violated, because testing for HWE is irrelevant, or because of some other reason.

Some studies included in the systematic review described analysis techniques to overcome the limitations of the case-only design. These included using multivariable modeling in the analysis to control for a third factor causing non-independence of genetic and environmental factors (e.g., family history of disease), though this approach required that genotype and exposure were independent at each level of the third covariate,(23,55) and using Bayes-type models in which prior information was incorporated into the case-only analysis.(24,30,39) Two of these Bayesian models derived an interaction estimator that was weighted towards a case-only analysis if evidence for gene-environment independence among controls was strong and toward a case-control analysis otherwise.(24,30) Similarly, Chen et al (22) tested an approach in which a case-only analysis was done in the first stage, followed by a smaller-scale case-parent/case-sibling study. These methods take advantage of the statistical efficiency of the case-only estimate and the unbiased properties of the case-control or case-parent/case-sibling estimates.

Other included studies evaluated the use of the case-only design in novel settings or derived a novel method of analyzing case-only data. For example, in large randomized clinical

trials with failure time end points, Vittinghoff and Bauer(42) showed that interactions between treatment and baseline covariates could be measured using only the trial participants who experienced the outcome. This case-only analysis gave results similar to a Cox regression analysis of the full dataset and was less biased than a case-cohort analysis of the dataset, provided that overall event rates were ≤20% and early drop-out rates were either non-differential by treatment and subgroup or less than 10%. Examples of novel analysis techniques included incorporating a third genetic or environmental factor in the analysis to study three-way gene-environment interactions,(55) and derivation of a polytomous logistic regression model that allowed genetic interaction to be assessed in studies using discrete or continuous traits.(56)

In interpreting our findings it is necessary to keep in mind potential limitations of this investigation. First, because the case-only design is poorly indexed, it was difficult to identify studies to include, and our search strategy may have missed some evaluations. Selective outcome reporting may have biased the results of the meta-regression analysis if interactions in which case-only and case-control estimates were discrepant were not reported. However it was impossible to identify any such studies. The funnel plot (Appendix F) suggests a lack of small studies in which the case-control estimate was larger than the case-only estimate. On the other hand, small studies in which the opposite was true are not missing and there is no reason to believe that the publication bias would favour a particular direction.

Another limitation of the meta-regression analysis was the small number of included studies. This could have limited the ability to detect significant differences in the IOR_{CC}/IOR_{CO} ratio attributable to methodological characteristics. The studies that used data from a secondary source may have selected their data to support their theoretical arguments, and several authors acknowledged selecting interactions from the secondary dataset on this basis. Nonetheless, the

IOR_{CC}/IOR_{CO} ratio calculated from the fifteen studies that used primary data (0.98, 95% CI 0.80-1.21) did not differ significantly from the overall ratio. Imputing missing variances using the mean variance of included studies could also have biased the meta-regression analysis if the information was not missing at random. This assumption appeared to be valid however, since all contacted authors responded and showed willingness to provide requested 2x4 tables. Data had been lost in one case and the authors were lost to subsequent follow up in two cases. Furthermore, imputation of variance based on the arithmetic mean variance in meta-analysis has been shown to give results similar to the true effect and to those computed using multiple imputations.(57) Modeling the largest IOR_{CC}/IOR_{CO} ratio when more than one interaction was eligible ensured that the meta-analysis did not under-estimate the difference between the case-only and case-control methods. A sensitivity analysis using the smallest ratios was initially intended but was not undertaken since no significant difference was found using the largest ratios.

In conclusion, this systematic review confirmed that the validity of the case-only design for the study of gene-environment interaction depends on the independence of genetic and environmental factors in the source population. The same principle applies to genetic factors in studies of gene-gene interaction. Limited evidence to date suggests that independence at the population level may be likely and, because the case-only study is typically used when the disease is rare, tests of gene-environment association in controls may provide a valid approximation of the association in the source population. If non-independence is suspected and can be quantified, then a model incorporating the source of non-independence can be used. When comparing case-only and case-control interaction odds ratios, it is important to realize that, depending on how controls were selected, different population parameters are estimated by

both study designs. Depending on the disease under study, this can result in differences in the calculated odds ratios. The meta-regression analysis suggested that this difference was not common in practice, although it did not establish whether the case-only or case-control estimates were unbiased relative to the true interaction effect. Although theoretical biases in the case-only design are possible, as improvements are made in understanding of the relationships between genes and environmental exposures in the population, the case-only design may prove to be a valuable tool in the search for gene-environment interactions in disease etiology.

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3. Breast cancer risk in relation to alcohol consumption and *BRCA* gene mutations – A case-only study of gene-environment interaction

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3.1. Abstract

BACKGROUND: Mutations in the *BRCA1* and *BRCA2* genes confer a significantly increased lifetime risk of breast cancer. The variable penetrance of these genes suggests that other genetic or environmental factors may interact with these mutations to modify breast cancer risk. The objective of this study was to measure departures from multiplicative effects of alcohol consumption and *BRCA* gene mutations.

METHODS: A nested case-only study of breast cancer was carried out in a French-Canadian population tested for *BRCA* gene mutations. Participants completed a food frequency and lifestyle risk factor questionnaire. Alcohol consumption was dichotomized at the median and the case-only interaction odds ratio (IOR_{CO}) was calculated.

RESULTS: A total of 857 women, including 10 *BRCA1* mutation carriers and 33 *BRCA2* mutation carriers, participated in the study. No significant interaction between alcohol consumption and *BRCA1* mutations was detected, although the interaction with wine consumption was suggestive of a less than multiplicative effect (IOR_{CO} =0.38, 95% CI 0.08-1.81). Consumption of alcohol other than wine interacted significantly with *BRCA2* mutations (IOR_{CO} =2.15, 95% CI 1.03-4.49).

CONCLUSIONS: The sub- multiplicative effect may be compatible with a protective effect of wine consumption among BRCA1 mutation carriers, possibly through the action of resveratrol. Women with *BRCA2* gene mutations may be at greater risk of alcohol-induced breast cancer than are women without *BRCA* gene mutations.

3.2. Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer deaths among Canadian women. The 2009 projected incidence and mortality rates in Canada are 101.9 per 100,000 and 22.0 per 100,000 respectively.(1) Inherited mutations in the *BRCA1* or *BRCA2* genes confer a significantly increased lifetime risk of breast cancer, with the time of onset often occurring at an early age. In a meta-analysis of 22 studies of *BRCA1* and *BRCA2* mutation carriers unselected for family history, the average cumulative risk of breast cancer by 70 years of age was 65% (95% CI 44%-78%) in *BRCA1* carriers and 45% (95% CI 31%-56%) in *BRCA2* carriers.(2) Given the incomplete penetrance of these mutations, other genetic and environmental factors may interact with *BRCA* gene mutations as determinants of cancer risk. Understanding these factors could have important implications for breast cancer prevention.

Based on a systematic review of the worldwide epidemiological evidence on alcohol consumption and breast cancer risk, the 2007 World Cancer Research Fund report (3) concluded, that "the evidence that alcoholic drinks are a cause of premenopausal and postmenopausal breast cancer is convincing". The increase in risk for every additional 10g of alcohol consumed on a daily basis was estimated to be in the range of 6 to 10%. Moreover, it has been estimated that approximately 4% of breast cancers in developed countries may be attributable to alcohol consumption.(4) Two studies that compared alcohol consumption patterns among affected and unaffected *BRCA* mutation carriers have reported null associations between alcohol consumption and breast cancer risk.(5,6) To our knowledge, no study has compared the risk of breast cancer associated with alcohol consumption among mutation carriers and non-carriers within the same study population.

The case-only design has been proposed as an efficient method to measure departure from multiplicative gene-environment interactions.(7,8) The design avoids problems with control group selection and, by elimination of control group variability, has substantially more power to detect interaction than does a case-control study.(9) It is important to note, however, that the validity of the case-only design depends on the independence of genotype and environment in the source population.(10) The objective of the present study was to determine if there exists an interaction between alcohol consumption and mutations in the *BRCA1* or *BRCA2* genes using a case-only design nested within an ongoing genetic epidemiology study of breast cancer.

3.3. Methods

3.3.1. Study Population

Study participants were recruited between October, 2004 and March, 2009 from an outpatient breast clinic at the Centre hospitalier de L'Université de Montréal (CHUM), Hôtel-Dieu and have been described previously.(11,12) Eligible women were of French-Canadian ancestry, between 25 and 65 years of age, and diagnosed with their first pathologically-confirmed breast cancer within the last ten years. Cases were defined as: (i) those diagnosed before 50 years of age with ductal carcinoma in situ (DCIS) and a family history of breast/ovarian cancer; (ii) those diagnosed before 50 years of age with invasive breast cancer; and (iii) those with a family history of breast/ovarian cancer diagnosed after 50 years of age with invasive breast cancer or DCIS. Family history was defined as two or more first or second degree relatives with breast and/or ovarian cancer. At the time of study enrolment, women received counselling and provided written consent for genetic testing for *BRCA* gene mutations.

When a *BRCA* mutation was identified in a proband, genetic testing was offered to other at-risk women in her family. These women were also invited to participate in the study, regardless of age and time between diagnosis and interview. This study was approved by the institutional research ethics board of the CHUM.

3.3.2. Genetic Testing

Genomic DNA was extracted from peripheral blood leukocytes or saliva using Puregene DNA Isolation Kits (Gentra Systems, Minneapolis, USA). Multiplex PCR amplification followed by fragment analysis was used to screen for 2953delGTA/insC in *BRCA1* and for 3398delAAAAG and 8765delAG in *BRCA2*. (The primer sequences are available upon request.) All forward primers were 50 fluorescently labelled with Cy5. Forty nanograms of DNA were amplified with PCR conditions of an initial denaturation at 94°C for 12 min followed by 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 1 min. Multiplex PCR products were electrophoresed at 70 W constant power for 90 min on a 6% denaturing polyacrylamide gel, followed by wet gel scanning with a PhosphorImager (Amersham Biosciences, Piscataway, NJ, USA).

PCR restriction fragment length polymorphism (PCRRFLP) was used to screen for the C4446T mutation in *BRCA1* and the G6085T mutation in *BRCA2*. For the detection of C4446T, a modified forward primer was designed to create a restriction site for the enzyme AciI in the amplicons by introducing a base substitution (T > C) at the -2 position relative to the target mutation site. For the detection of G6085T, a modified reverse primer was used to create a restriction site for the enzyme GsrGI in the amplicons by introducing a base substitution (A > C) at the +2 position relative to the target mutation site. For both mutation detections, the PCR

cycling profile was the same as that described above. PCR products were incubated with the corresponding restriction enzyme at 37°C overnight. PCR product with the C4446T mutation was undigested by AciI (New England BioLabs, Ipswich, MA, USA) or was digested into 125 and 19 bp fragments in the case of a wild type. PCR product with the G6085T mutation was digested by GsrGI (New England BioLabs), which gave two fragments of 93 and 25 bp, whereas the wild type was undigested. Fragments were resolved by 2.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The six mutations tested in this population represent 86% of mutations in the *BRCA1/2* genes in French-Canadian hereditary breast and/or ovarian cancer families.(13)

3.3.3. Assessment of Alcohol Consumption

Cases completed an interviewer-administered food frequency questionnaire (FFQ) developed by the National Cancer Institute of Canada that asked about consumption patterns in the year prior to breast cancer diagnosis. This instrument has been tested for validity and reproducibility, (14) and has been employed in several studies of diet and breast cancer.(5,15) The FFQ specified the type of alcohol and volume as follows: beer (1 bottle/can), wine (5oz./140ml glass), sherry/port/etc. (3oz./85ml glass), and spirits (1.5oz. /45ml drink). Subjects were asked how many of each beverage type they consumed on a daily, weekly, or monthly basis, and for how many months of the year they consumed these beverages. These data were used to construct a continuous measure of alcohol intake defined in terms of the number of drinks per week.

3.3.4. Assessment of Other Lifestyle Factors

Participants also completed an interviewer-administered lifestyle risk factor questionnaire that included items related to ethnicity, family history, reproductive and medical histories, menopausal status, smoking habits, oral contraceptive use, and hormone replacement therapy (HRT) use. Subjects reported their weight at 20, 30, and 40 years of age, as well as their maximum attained weight and the age at which they reached this weight. The most recent weight value before breast cancer diagnosis was used to calculate body mass index (BMI). Oophorectomy was defined as partial or complete removal of one or more ovaries.

3.3.5. Statistical Methods

Differences in continuous and categorical variables were compared by Student's t-test and by the Chi-square test, respectively. Where 25% of the cells had expected counts less than 5, p-values based on Fisher's exact test rather than the Chi-square test were reported.

Interaction was assessed separately for the *BRCA1* and *BRCA2* genes using non-mutation carriers as the comparison group. Alcohol was dichotomized at the median intake of all cases. Unconditional univariable and multivariable logistic regression were used to calculate the case-only interaction odds ratio (IOR_{CO}). The IOR_{CO} measures the extent to which the joint effect of genotype (*BRCA1/2* status) and environment (alcohol consumption) differs from the product of the independent effects of genotype alone and environment alone. IOR_{CO} values greater than unity indicate a supra-multiplicative interaction between genotype and environment while IOR_{CO} values less than unity indicate sub-multiplicative effects.(8) All multivariable models were adjusted for age at diagnosis. The effects of other potential confounders on the IOR_{CO} were tested by stratified analyses. All analyses were done with SAS 9.1 (SAS Institute, Cary, NC).

3.4. Results

Of the 954 eligible subjects, all agreed to participate and provided a blood (935 cases) or saliva (19 cases) sample, all of which were genotyped. A total of 895 patients completed the nutrition and lifestyle risk factor questionnaires. Information on the date of breast cancer diagnosis was missing or inconsistent for 23 patients, as was information on alcohol consumption in 15 patients, resulting in a final sample of 857 women, including 10 women with a *BRCA1* mutation and 33 women with a *BRCA2* mutation, from 823 families. Affected individuals in each family were either first-, second-, third-, or fourth-degree relatives (occurring within the same branch of the family) of the index case who was selected for mutation analysis.

The mean time between diagnosis and interview was 3.1 years (range <1 month to 24.7 years). Subjects with and without *BRCA* gene mutations were similar with respect to age at interview, time between diagnosis and interview, BMI, parity, age at menarche, menopausal status, age at menopause, oral contraceptive use, HRT use, tamoxifen use, history of mastectomy and oophorectomy, and smoking (Table 3-1). The age at breast cancer diagnosis was significantly lower for women with a *BRCA* gene mutation than for non-mutation carriers (44.7 years of age vs. 48.0 years of age, p=0.006), and significantly more mutation carriers reported a family history of breast/ovarian cancer (90.7% vs. 75.6%, p=0.02). The proportion who reported that they ever drank alcohol in the year prior to breast cancer diagnosis was similar between mutation carriers and non-carriers (p=0.12).

Table 3-1. Characteristics of study participants with and without *BRCA* gene mutations.

Characteristic	Non-mutation carriers (n=814)	Mutation Carriers (n=43)	p-value[a]
Mean year of birth	1955.0	1957.6	0.10
Mean age at diagnosis, years	47.9	44.7	0.006
Mean time between diagnosis and interview, years	3.1	3.7	0.46
Mean age at interview, years	51.0	48.4	0.10
Premenopausal, N (%)			
Yes	388 (48.0)	25 (58.1)	0.19
Mean age at menarche	12.6	12.9	0.18
Ever oral contraceptive use, N (%)			
Yes	747 (91.8)	41 (95.4)	0.57
Ever smoked, N (%)			
Yes	515 (63.3)	31 (72.1)	0.24
Oophorectomy, N (%)			
Yes	79 (10.0)	6 (14.3)	0.43
Mean age at menopause, years	45.7	43.6	0.16
Ever HRT use, N (%)			
Yes	193 (23.7)	8 (18.6)	0.44
Mean BMI	24.5	23.8	0.33
Mean parity	1.3	1.6	0.08
Tamoxifen use, N (%)			
Yes	11 (1.4)	1 (2.3)	0.46
Mastectomy, N (%)			
Yes	0	0	-
Family history of breast/ovarian cancer, N (%)			
Yes	607 (75.6)	39 (90.7)	0.02
Alcohol consumption in year prior to diagnosis, N (%)			
Yes	687 (84.4)	40 (93.0)	0.12

[a]p-value for difference between mutation carriers and non-mutation carriers (based on Student's t-test for continuous variables and Chi-square or Fisher's exact test for binary variables).
HRT=Hormone replacement therapy; BMI=Body mass index

All *BRCA1* mutation carriers consumed alcohol in the year prior to breast cancer diagnosis, compared to 84.4% of non-mutation carriers (p=0.38). However, women with *BRCA1* mutations consumed less total alcohol and less wine than did women without *BRCA* mutations (Table 3-2). None of the interactions between alcohol consumption and *BRCA1* were significant

(Table 3-3), although the IOR_{CO} for total alcohol consumption was suggestive of a less than multiplicative effect (IOR_{CO} =0.79, 95% CI 0.22-2.83). This effect was restricted to wine consumption (IOR_{CO} =0.38, 95% CI 0.08-1.81) and not to consumption of other alcohol types (IOR_{CO} =2.49, 95% CI 0.64-9.73). Stratification by potential confounders did not reveal evidence of effect modification, although the sample sizes were small (Appendix G).

Among women with *BRCA2* mutations, 90.9% consumed alcohol in the year prior to breast cancer diagnosis, which did not differ significantly from the proportion of non-mutation carriers who had consumed alcohol (84.4%, p=0.31). *BRCA2* mutation carriers consumed more total alcohol than did women without *BRCA* mutations (Table 3-2). The IOR_{CO} for total alcohol consumption was suggestive of a supra- multiplicative effect (IOR_{CO} =1.98, 95% CI 0.96-4.10), which was significant for consumption of other alcohol types (IOR_{CO} =2.15, 95% CI 1.03-4.49), but not for wine consumption (IOR_{CO} =0.95, 95% 0.91-1.00) (Table 3-3). A significant supra-multiplicative interaction between total alcohol consumption and *BRCA2* mutations was also observed among women whose BMI was 25 or greater (IOR_{CO}=5.72, 95% CI 1.19-27.42), but not among women whose BMI was less than 25 (IOR_{CO}=1.24, 95% CI 0.53-2.87); a significant supra-multiplicative interaction was also observed among parous women (IOR_{CO}=2.31, 95% CI 1.05-5.05) but not nulliparous women (IOR_{CO}=0.87, 95% CI 0.12-6.23) (Appendix G).

Table 3-2. Alcohol consumption patterns in the year prior to breast cancer diagnosis in non-mutation carriers, *BRCA1* mutation carriers, and *BRCA2* mutation carriers.

Alcohol Consumption Patterns in Year Prior to Diagnosis	Non-Carriers (n=814)	*BRCA1* Carriers (n=10)	*BRCA2* Carriers (n=33)
Consumed alcohol, N (%)	687 (84.4)	10 (100)	30 (90.9)
Median number of drinks consumed per week, (IQR)			
Total Alcohol	3.0 (0.5-7.0)	2.5 (1.8-4.8)	5.0 (1.5-7.5)
Wine	2.0 (0.3-5.0)	1.0 (0.5-2.0)	3.0 (1.0-5.0)
Other Alcohol (beer, fortified wine, spirits)	0.3 (0.0-1.5)	0.9 (0.0-2.0)	0.7 (0.1-2.0)

IQR=Interquartile range

Table 3-3. Interaction between alcohol consumption in drinks/week (dichotomized at the median of all cases) and *BRCA1* and *BRCA2* gene mutations.

Model	≤median (No. non-carriers / No. carriers)	>median (No. non-carriers / No. carriers)	p-value[a]	IOR_{CO} (95% CI) (unadjusted)	IOR_{CO} (95% CI) (adjusted)[b]
BRCA1					
Total Alcohol (median=3 drinks/week)	423/6	391/4	0.75	0.72 (0.20-2.58)	0.79 (0.22-2.83)
Wine (median=2 drinks/week)	478/8	336/2	0.21	0.36 (0.08-1.69)	0.38 (0.08-1.81)
Other Alcohol[c] (median=0.33 drinks/week)	420/3	394/7	0.21	2.49 (0.64-9.69)	2.49 (0.64-9.73)
BRCA2					
Total Alcohol (median=3 drinks/week)	423/12	391/21	0.08	1.89 (0.92-3.90)	1.99 (0.96-4.11)
Wine (median=2 drinks/week)	478/16	336/17	0.24	1.51 (0.75-3.03)	1.58 (0.78-3.17)
Other Alcohol[c] (median=0.33 drinks/week)	420/11	394/22	0.04	2.13 (1.02-4.45)	2.15 (1.03-4.50)

[a]Based on Chi-squared or Fisher's exact test

b Adjusted for age at diagnosis

[c]Includes beer, fortified wine, and spirits

Restricting analyses to the 728 cases who had consumed alcohol in the year prior to diagnosis did not change the overall trends, although the interaction between alcohol consumption other than wine and *BRCA2* was no longer significant (Appendix H).

3.5. Discussion

This study found no significant departure from a multiplicative interaction between higher amounts of alcohol consumption and *BRCA1* gene mutations, although the results were suggestive of a less than multiplicative effect of wine consumption and *BRCA1* mutations. These results may be compatible with a protective effect of wine consumption in *BRCA1* mutation carriers, although they could also be compatible with a protective effect of wine consumption in *BRCA1* mutation carriers and non-carriers, or a detrimental effect of wine consumption among non-mutation carriers only. A significant supra- multiplicative effect of total beer, fortified wine, and spirit consumption and *BRCA2* mutations was observed, meaning that women with *BRCA2* mutations who consume higher amounts of alcohol other than wine may be at greater risk of breast cancer than women without *BRCA* mutations who consume higher amounts of alcohol other than wine. The joint effect of alcohol consumption and *BRCA2* mutations was greater in women with a high BMI and in parous women.

Wine is rich in cancer-fighting polyphenols,(16,17) and some studies on women in the general population have found a null or even protective effect of wine consumption on breast cancer risk. A population-based case-control study in southern France found that low (<10-12 g/day) and regular (5 times/week or more) wine consumption reduced the risk of breast cancer, compared to no or sporadic wine consumption.(18) In a population-based case-control study

conducted in the U.S., breast cancer risk was associated with distilled spirit consumption, but with neither red nor white wine consumption.(19) In the U.S. Women's Health Study of 39,876 female health professionals followed for an average of 10 years, consumption of red wine was not associated with breast cancer risk.(20) In contrast, the Million Women Study in the United Kingdom,(21) as well as a pooled analysis of 6 prospective cohort studies conducted in Canada, the Netherlands, Sweden, and the U.S., (22) found that consumption of all types of alcohol increased the risk of breast cancer.

There is some evidence that resveratrol, produced by grape vines in response to injury and found especially in red wine, may mediate the inverse association between breast cancer and wine consumption.(17,23) In vitro, resveratrol inhibits tumor initiation and progression by inducing cell cycle arrest and apoptosis.(24-26) The protective effects of resveratrol may be particularly evident in *BRCA1* mutation carriers. In human breast tumor cell lines, resveratrol binds to the estrogen receptor and up-regulates transcription of *BRCA1* and of genes whose proteins interact with *BRCA1*.(26,27) In in vitro and mouse models, resveratrol is a potent inhibitor of the initiation and progression of *BRCA1* mutant tumors.(25)

There are several potential mechanisms by which alcohol consumption may increase breast cancer risk, including through the disruption of estrogen metabolism and response.(28) Higher levels of circulating estrogens have been associated with alcohol consumption, (29-31) and in vitro, ethanol increases cellular responsiveness to estrogen.(32) Accordingly, alcohol consumption may be more important in the etiology of estrogen receptor positive (ER+) tumors than estrogen receptor negative (ER-) tumors.(33,34) Approximately 80% of tumors in *BRCA2* mutation carriers are ER+, compared to less than 70% of tumors in non-mutation carriers and 20% of tumors in *BRCA1* mutation carriers.(35) This difference could explain the greater than

multiplicative effect of alcohol consumption and *BRCA2* mutations, but not *BRCA1* mutations, observed in this study.

An earlier study by our group (5) that included 89 affected and 48 unaffected French-Canadian *BRCA* mutation carriers failed to find an association between alcohol consumption in the year prior to diagnosis and breast cancer risk, regardless of alcohol type, although results were not reported separately for *BRCA1* and *BRCA2* mutation carriers. A study of mutation carriers less than 50 years of age from the United States, Canada, and Australia found no association between alcohol consumption and breast cancer risk among 195 affected and 302 unaffected *BRCA1* carriers.(6) Among 128 affected and 179 unaffected *BRCA2* mutation carriers, an inverse association with modest alcohol consumption (1-4 g/day) was observed, as compared to no alcohol consumption. The difference between these findings and the results of the current analysis could be due to differences in the comparison group, or due to the older age at diagnosis among participants in the current study, since alcohol consumption may be more strongly associated with breast cancers diagnosed at a later age.(4,36).

As tumor suppressor proteins, *BRCA1* and *BRCA2* are integral to genome stability.(37-39) In response to DNA damage, *BRCA1* is phosphorylated, triggering cell cycle arrest. *BRCA1* also acts indirectly in the repair of double-strand breaks (DSB) by homologous recombination (HR) through several different processes. Conversely, *BRCA2* participates directly in the repair of DSB and is an essential part of the HR machinery. These distinct cellular functions of *BRCA1* and *BRCA2* likely explain the differences in estrogen receptor status, histopathological characteristics, and gene expression profiles of tumors arising in *BRCA1* and *BRCA2* mutation carriers.(37) The effects of lifestyle and hormonal factors on breast cancer risk may also depend

on which gene is mutated.(40) For these reasons, it was important to consider *BRCA1* and *BRCA2* mutation carriers separately.

A necessary assumption of the case-only design is that genotype and environment are independent of each other within the population from which cases were selected. This assumption could have been violated in the present study if subjects with a family history of breast/ovarian cancer were less likely to consume alcohol. If the source of such non-independence can be measured, it can be controlled for by including it as a covariate in the multivariable model.(41,42) All *BRCA1* mutation carriers had a family history of breast/ovarian cancer, preventing its inclusion in the model. Adjustment for family history among *BRCA2* mutation carriers did not appreciably change the results (Appendix I), suggesting that bias caused by non-independence of *BRCA* mutations and alcohol consumption was unlikely. The case-only study also only measures departure from multiplicative interactions and not from additive effects. Although the concept of interaction in epidemiology is often debated,(43) departures from multiplicative models are appropriate for many biologically plausible modes of gene-environment interaction.(44)

This study is also limited by the low prevalence of *BRCA* gene mutation carriers and by the lack of historical information on alcohol consumption. The FFQ only asked about consumption patterns in the year prior to breast cancer diagnosis. Nonetheless, recent consumption may be most relevant to breast cancer risk.(36,45,46) The FFQ also did not differentiate between red and white wine. Fortified wines, made by adding additional alcohol to wine, were grouped with beers and spirits in the analysis because, relative to all other alcohol types, they have they highest concentration of the carcinogen acetyaldehyde (47) and non-detectable amounts of resveratrol.(48)

Survival bias among cases who were interviewed several years after breast cancer diagnosis is a possibility if alcohol consumption is associated with reduced survival. However, there was little difference in the results between cases who were interviewed less than and more than 3 years after diagnosis, suggesting that survival bias is not a major concern.

In conclusion, this study found a less-than multiplicative effect of wine consumption and *BRCA1* gene mutations. This finding may be compatible with a protective effect of wine consumption among BRCA1 mutation carriers, perhaps through the action of resveratrol. Higher amounts of alcohol consumption may act synergistically with *BRCA2* gene mutations to increase the risk of breast cancer. These results should be confirmed in larger population-based studies of breast cancer patients tested for *BRCA* gene mutations, as well as in studies of affected and unaffected *BRCA* mutation carriers. Future studies should distinguish between red and white wine consumption.

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4. Alcohol Consumption and the Risk of Breast Cancer among *BRCA1* and *BRCA2* Mutation Carriers

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4.1. Abstract

BACKGROUND: Alcohol consumption increases the risk of breast cancer among women in the general population, but its effect on women who carry a *BRCA1* or *BRCA2* mutation is unclear.

METHODS: We conducted a case-control study of 1,925 matched pairs of women who carry a *BRCA1* (n = 1,480 pairs) or a *BRCA2* (n = 445 pairs) mutation to investigate the relationship between alcohol consumption and breast cancer risk. Information on alcohol consumption, including the number of drinks consumed per week on average and the type of alcohol regularly consumed, was obtained from a questionnaire administered during the course of genetic counselling or at the time of enrolment.

RESULTS: A modest inverse association between breast cancer and reported current alcohol consumption was observed among women with a *BRCA1* mutation (OR = 0.82, 95% CI 0.70-0.96), but not among women with a *BRCA2* mutation (OR = 1.00; 95% CI 0.71-1.41). Compared to non-drinkers, exclusive consumption of wine was associated with a significant reduction in the risk of breast cancer risk among *BRCA1* carriers (p-trend = 0.01).

CONCLUSIONS: Alcohol consumption does not appear to be associated with an increased risk of breast cancer in women carrying a *BRCA1* or *BRCA2* mutation. A possible protective effect of wine consumption among women with a *BRCA1* mutation warrants further study.

4.2. Introduction

Inherited mutations in the *BRCA1* and *BRCA2* genes confer significantly increased lifetime risks of breast cancer: in a meta-analysis of 22 studies of *BRCA1* and *BRCA2* mutation carriers who were unselected for family history, the average cumulative risk of breast cancer up to 70 years of age was 65% (95% CI 44%-78%) in *BRCA1* carriers and 45% (95% CI 31%-56%) in *BRCA2* carriers.(1) Given the high risk of breast cancer in these women, it is important to find potentially modifiable risk factors that may be integrated into a cancer prevention program.

Alcohol consumption is an established risk factor for breast cancer. Most epidemiological studies have found a dose-dependent increase in breast cancer risk beginning at consumption levels as low as one drink per day.(2-8) However, participants in these studies were women from the general population who were unlikely to carry a mutation in the *BRCA1* or *BRCA2* genes. The characteristics of the breast cancers that arise in women with *BRCA1* or *BRCA2* mutations may differ from those of women in the general population and the risk factor profile may be different as well. The objective of this study was to determine if the risk of breast cancer is influenced by alcohol consumption in women with a *BRCA1* or *BRCA2* mutation.

4.3. Materials and Methods

4.3.1. Study Population and Design

Eligible women were identified from 54 participating centres in eight countries. These women were participants in ongoing research protocols at the host institutions. The majority of study participants received counselling and provided written informed consent for genetic testing. The institutional review boards of the host institutions approved the study.

In most cases, genetic testing was initially offered to women who had been diagnosed with breast or ovarian cancer. When a *BRCA1* or *BRCA2* mutation was identified in a proband or her relative, genetic testing was offered to other at-risk individuals in the family. Mutation detection was performed using a range of techniques, but all abnormal nucleotide sequences were confirmed by direct sequencing of DNA. A woman was eligible for the current study when molecular analysis established that she was a carrier of a known deleterious mutation in the *BRCA1* or *BRCA2* gene. Most (>95%) of the mutations identified in the study subjects were either non-sense mutations, deletions, insertions, or small frameshifts resulting in premature termination of the protein. Women with variants of unknown significance were not included.

Information on cancer history was available for 8,192 women. Potential subjects were excluded if they had been diagnosed with ovarian cancer (1,043 women) or another cancer (681 women), or if information was missing on alcohol consumption (175 women) or on another key variable (87 women). Case subjects were women with a diagnosis of invasive breast cancer. Control subjects were women who were never diagnosed with breast cancer and who were carriers of a mutation in either *BRCA1* or *BRCA2* (or both). After exclusions, there were 6,206 eligible women, including 2,707 with breast cancer (potentially eligible cases) and 3,503 without breast cancer (potentially eligible controls).

Each case was matched with a single control subject according to year of birth (within one year), mutation in the same gene (*BRCA1* or *BRCA2*), and country of residence. Within Canada, women were also matched on ethnicity (French-Canadian or non-French-Canadian). A control was eligible to be matched to a given case if the date of interview or date of prophylactic mastectomy in the matched control occurred at or after the year of breast cancer diagnosis in the

case subject. A total of 1,925 matched case-control pairs was generated, including 1,480 pairs with *BRCA1* mutations and 445 pairs with *BRCA2* mutations.

4.3.2. Data Collection and Definition of Variables

Case and control subjects completed a questionnaire between January, 1992 and February, 2009. The questionnaire included items related to ethnicity, family history, reproductive and medical histories, menopausal status, smoking, oral contraceptives and hormone replacement therapy. Subjects were asked whether or not they drank alcoholic beverages and, if so, to estimate the number of drinks they consumed per week on average (0-3, 4-9, 10-20, or 20 or more). From 2007 onwards, the questionnaire included an item for the type of alcohol regularly consumed (wine, beer, or liquor; subjects could select more than one). Subjects reported their weight at 20, 30, and 40 years of age, as well as their maximum attained weight and the age at which they reached this weight. The most recent recorded body weight before breast cancer diagnosis was used to calculate body mass index (BMI) in the case; body weight at the corresponding age was used to determine BMI in the matched control. Self-administered questionnaires were given to the subjects during clinical appointments at the individual study centers or were mailed to the study subject's home at a later date.

4.3.3. Statistical Methods

The distributions of continuous and categorical variables were compared between case and control subjects using Student's *t*-test and the Chi-square test, respectively. Odds ratios for breast cancer were estimated with regard to current alcohol consumption (yes/no) and to the number of drinks consumed per week. Analyses were stratified by mutation (*BRCA1 vs BRCA2*) age at diagnosis (<50 *vs* ≥50 years of age), by BMI (<25 *vs* ≥25), and by smoking (ever *vs* never). Odds ratios were estimated using conditional logistic regression. Odds ratios were also

calculated by type of alcohol typically consumed. All multivariable models were adjusted for ethnicity (French-Canadian, Jewish, other white, other), menopausal status, oral contraceptive use (ever/never), hormone replacement therapy (HRT) use (ever/never), smoking (ever/never), oophorectomy, BMI (<25, 25 to <30, ≥30), and parity (0, 1, 2, 3, 4+). All analyses were done with SAS Version 9.1 (SAS Institute, Cary, NC).

4.4. Results

Cases and controls were similar with respect to age at interview, menopausal status, past use of oral contraceptives, smoking, BMI, and parity (Table 4-1). Controls were significantly more likely than cases to have to have had an oophorectomy (10.7% *vs* 8.7%, p = 0.04) and to have used hormone replacement therapy (10.8% *vs* 6.2%, p<0.0001). Among menopausal women, 24.9% of the cases and 44.0% of the controls had used hormone replacement therapy. Controls had a higher mean age at menopause than cases (44.8 *vs* 42.7, p = 0.0001); however, among women who had undergone natural menopause, the mean age of menopause was 47.8 years for cases and was 47.4 years for controls.

Table 4-1. Characteristics of study participants

Characteristic	Controls (N=1925)	Cases (N=1925)	p-value[a]
Country of study site, N (%)			
Austria	26 (1.4)	26 (1.4)	
Canada, not French-Canadian	430 (22.3)	430 (22.3)	
French Canadian	141 (7.3)	141 (7.3)	
Israel	62 (3.2)	62 (3.2)	
Italy	6 (0.3)	6 (0.3)	
Norway	45 (2.3)	45 (2.3)	
Poland	600 (31.2)	600 (31.2)	
UK	7 (0.4)	7 (0.4)	
USA	608 (31.6)	608 (31.6)	
Mutation, N (%)			
BRCA1	1,480 (76.8)	1,480 (76.8)	
BRCA2	445 (23.1)	445 (23.1)	
Mean year of birth	1955.9	1955.8	
Mean age at diagnosis, years	40.3	40.3	
Mean time between diagnosis and interview, years	6.8	6.3	
Mean age at interview, years	47.0	46.6	0.13
Ethnicity, N(%)			
French-Canadian	133 (6.9)	150 (7.8)	
Jewish	359 (18.7)	284 (14.8)	
Other	31 (1.6)	55 (2.9)	
Other White	1,402 (72.8)	1,436 (74.6)	0.0008
Premenopausal, N (%)			
Yes	1,545 (81.7)	1,526 (80.2)	0.24
Ever oral contraceptive use, N (%)			
Yes	1,194 (62.3)	1,163 (60.6)	0.29
Ever smoked, N (%)			
Yes	829 (43.2)	833 (43.3)	0.93
Oophorectomy, N (%)			
Yes	205 (10.7)	167 (8.7)	0.04
Mean age at menopause, years	44.8	42.7	0.0001
Ever HRT use, N (%)			
Yes	206 (10.8)	119 (6.2)	<0.0001
Mean BMI	24.1	24.4	0.07
Mean parity	2.0	1.9	0.52

[a]p-value for difference between breast cancer cases and controls (based on Student's t-test for continuous variables and Chi-square test for binary variables).
HRT=Hormone replacement therapy; BMI=Body mass index

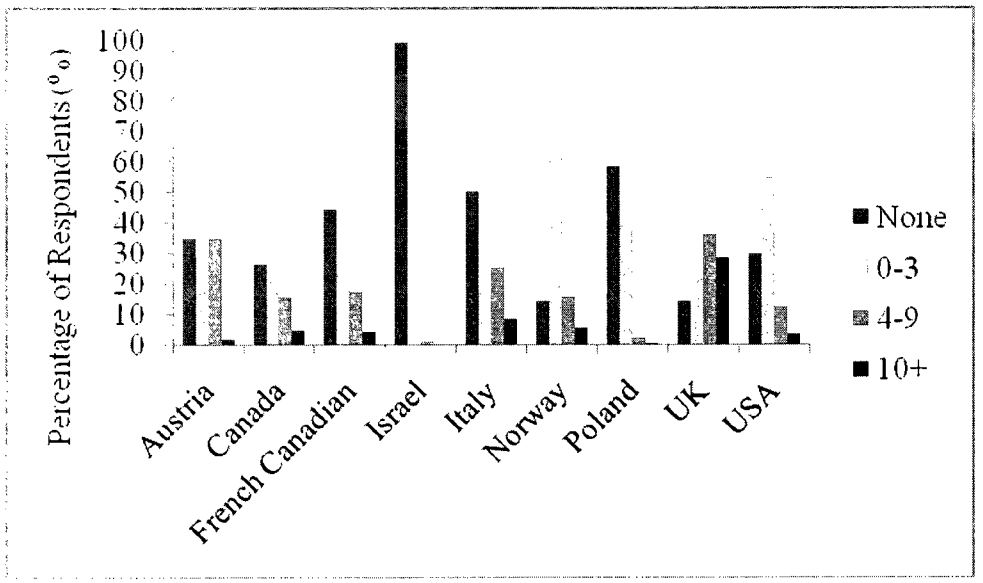
Current alcohol consumption was more commonly reported among controls than among cases (60.9% *vs* 57.3%, p<0.02) (Table 4-2). Overall, fewer than 3% of participants consumed 10 or more drinks per week. Average alcohol consumption varied widely by country, with Israel having the lowest average consumption (99% non-drinkers) and the United Kingdom the highest (29% of those from the United Kingdom consumed 10 or more drinks per week) (Figure 4-1). For some countries, the estimates were based on small numbers of women.

Table 4-2. Frequency of alcohol consumption and types of alcohol regularly consumed among cases and controls

Measure of Alcohol Consumption	**Controls (N=1,925)**	**Cases (N=1,925)**	**p-value[a]**
Current alcohol consumption, N(%)			
Yes	1,172 (60.9)	1,102 (57.3)	0.02
Number of drinks consumed per week, N (%)			
None	753 (39.1)	823 (42.8)	
0-3	916 (47.6)	849 (44.1)	
4-9	193 (10.0)	206 (10.7)	
≥10	63 (3.3)	47 (2.4)	0.04
Type of Alcohol, N (%)	**Controls (N=1,141)**	**Cases (N=1,141)**	
None	612 (53.6)	666 (58.4)	
Wine only	218 (19.1)	173 (15.2)	
Wine and other[b]	201 (17.6)	211 (18.5)	
Other[b]	110 (9.6)	91 (8.0)	0.02

[a]based on Chi-squared test
[b]Includes beer and spirits

Figure 4-1. Variation in the number of drinks consumed per week by country of study site



UK=United Kingdom; USA=United States of America

Among women with a *BRCA1* mutation, current alcohol consumption was more common among controls than among cases (58.7% *vs* 54.4%, p = 0.02). In a multivariable model, the odds ratio for breast cancer associated with current alcohol consumption was 0.82 (95% CI 0.70-0.96), and a significant trend of decreasing risk with increasing drinks per week was observed (p-trend = 0.03) (Table 4-3). When analyses were restricted to the 1034 pairs in which both the case and control consumed alcohol, the trend in multivariable odds ratios was no longer significant (p-trend=0.50) (Appendix J). The association was not appreciably modified by age at diagnosis or BMI, although the trend of decreasing risk with increasing drinks consumed per week was not significant among those who had ever smoked (Appendix K).

Among women with a *BRCA2* mutation, cases and controls were equally likely to consume alcohol (68.3% *vs* 66.7%, p = 0.62), with the multivariable odds ratio associated with current alcohol consumption estimated to be 1.00 (95% CI 0.71-1.41). The association between the number of drinks per week and breast cancer risk was not significant (p-trend = 0.72), nor

was it modified by restricting to pairs in which both the case and control consumed alcohol, by age at diagnosis BMI, or smoking.

Table 4-3. Association between breast cancer risk and the number of alcoholic drinks consumed per week among *BRCA1* and *BRCA2* mutation carriers

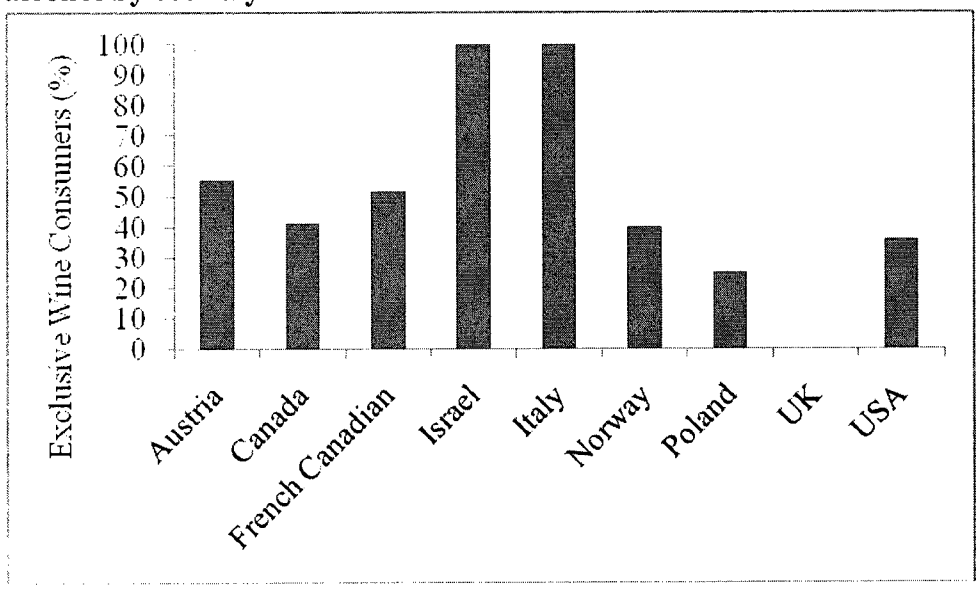
Model	Number of Alcoholic Drinks Consumed per Week				
	None	0-3	4-9	≥10	p-trend
BRCA1 (n=1480 pairs)					
Cases/controls	675/612	640/706	135/118	30/44	
Univariable OR (95% CI)	1.00	0.80 (0.68-0.94)	1.01 (0.76-1.35)	0.60 (0.37-0.97)	0.05
Multivariable OR (95% CI)[a]	1.00	0.77 (0.67-0.94)	0.98 (0.73-1.32)	0.55 (0.33-0.91)	0.03
BRCA2 (n=445 pairs)					
Cases/controls	148/141	209/210	71/75	16/18	
Univariable OR (95% CI)	1.00	0.94 (0.67-1.31)	0.90 (0.60-1.34)	0.85 (0.43-1.69)	0.53
Multivariable OR (95% CI)[a]	1.00	0.97 (0.67-1.41)	1.04 (0.67-1.63)	1.16 (0.55-2.45)	0.72

[a]Adjusted for ethnicity (other white, French-Canadian, Jewish, other), menopause, oral contraceptive use, HRT use, smoking, oophorectomy, BMI (<25, 25 to <30, ≥30), and parity (0, 1, 2, 3, 4+)
HRT=Hormone replacement therapy; BMI=Body mass index

The principal type of alcohol consumed was known for 1,141 case-control pairs. Thirty-nine percent of women who drank consumed wine exclusively, ranging from 0% to 100%, depending on the country (Figure 4-2). Among *BRCA1* carriers, consumers of wine exclusively had a reduced risk of breast cancer compared to non-drinkers (multivariable OR = 0.64, 95% CI 0.47-0.87), whereas women who did not consume wine exclusively had a risk of breast cancer that was similar to that of non-drinkers (multivariable OR = 0.89, 95% CI 0.70-1.12). A significant decreasing trend in the risk of breast cancer was seen with increasing weekly alcohol consumption for consumers of wine exclusively, but not for the other subgroups of types of alcohol consumed (Table 4-4). Among women with a *BRCA2* mutation, the odds ratios for

breast cancer associated with exclusive and non-exclusive wine consumption compared to no alcohol consumption were 1.01 (95% CI 0.61-1.69) and 0.88 (95% CI 0.53-1.48), respectively.

Figure 4-2. Prevalence of exclusive wine consumers among those who reported consuming alcohol by country



UK, United Kingdom; USA, United States of America

Table 4-4. Association between breast cancer risk and the number of alcoholic drinks consumed per week by the type(s) of alcohol typically consumed among *BRCA1* and *BRCA2* mutation carriers. The referent group is non-drinkers (543 *BRCA1* cases, 501 *BRCA1* controls; 123 *BRCA2* cases, 111 *BRCA2* controls).

Model	Number of Alcoholic Drinks Consumed per Week			
	0-3	4-9	≥10	p-trend
BRCA1 (n=895 pairs)				
Exclusive wine consumers				
Cases/controls	89/127	18/20	4/7	
Univariable OR (95% CI)	0.62 (0.45-0.85)	0.79 (0.40-1.56)	0.51 (0.15-1.77)	0.009
Multivariable OR (95% CI)[a]	0.62 (0.45-0.87)	0.82 (0.41-1.67)	0.39 (0.11-1.45)	0.01
Wine and other alcohol types[b]				
Cases/controls	126/114	35/27	7/7	
Univariable OR (95% CI)	1.02 (0.76-1.36)	1.15 (0.67-1.99)	0.89 (0.31-2.59)	0.61
Multivariable OR (95% CI)[a]	1.05 (0.78-1.42)	1.10 (0.61-1.96)	0.64 (0.21-1.99)	0.79
Other alcohol types[b]				
Cases/controls	60/80	10/9	3/3	
Univariable OR (95% CI)	0.69 (0.48-0.97)	1.05 (0.42-2.60)	0.89 (0.18-4.47)	0.16
Multivariable OR (95% CI)[a]	0.62 (0.43-0.91)	1.07 (0.40-2.85)	0.70 (0.13-3.75)	0.08
BRCA2 ***(n=246 pairs)***				
Exclusive wine consumers				
Cases/controls	41/43	18/17	3/4	
Univariable OR (95% CI)	0.86 (0.51-1.45)	1.04 (0.51-2.13)	0.63 (0.14-2.95)	0.68
Multivariable OR (95% CI)[a]	1.09 (0.60-1.95)	1.12 (0.49-2.60)	0.50 (0.08-3.02)	0.92
Wine and other alcohol types[b]				
Cases/controls	28/33	11/16	4/4	
Univariable OR (95% CI)	0.81 (0.43-1.51)	0.63 (0.28-1.43)	0.93 (0.23-3.79)	0.27
Multivariable OR (95% CI)[a]	0.90 (0.44-1.82)	0.81 (0.34-1.93)	1.38 (0.31-6.15)	0.68
Other alcohol types[b]				
Cases/controls	12/10	3/8	3/0	
Univariable OR (95% CI)	1.05 (0.42-2.61)	0.39 (0.10-1.55)	N/A	0.91
Multivariable OR (95% CI)[a]	1.19 (0.41-3.45)	0.54 (0.12-2.34)	N/A	0.79

[a]Adjusted for ethnicity (other white, French-Canadian, Jewish, other), menopause, oral contraceptive use, HRT use, smoking, oophorectomy, BMI (<25, 25 to <30, ≥30), and parity (0, 1, 2, 3, 4+)

[b]Includes beer and spirits

HRT=Hormone replacement therapy; BMI=Body mass index

4.5. Discussion

In this study, we found that increasing alcohol consumption was associated with a reduction in the risk of breast cancer among women with *BRCA1* mutations, but not among women with *BRCA2* mutations. This association was restricted to consumers of wine exclusively.

Contrary to what is seen in the general population, alcohol consumption did not increase breast cancer risk among participants in the current study, which may be explained by their young age at diagnosis, predominantly premenopausal status, and estrogen receptor (ER) status. In the general population, the association between alcohol consumption and breast cancer risk may be weaker among women diagnosed at a young age (2,9,10) and among premenopausal women (2,5,11) than among post-menopausal women. The reasons for this difference are unclear, but may reflect differences in the level of circulating ovarian hormones.(12,13) Alcohol consumption increases the levels of circulating estrogens (14-16) and has been more strongly associated with ER-positive tumors than with ER-negative tumors.(6,9) Among *BRCA1* carriers, approximately 20% of tumors are ER-positive.(17)

The modifying effect of consumption of specific types of alcohol on breast cancer risk among women in the general population is unclear. Some studies have found no difference in risk by alcohol type, (3,5,7,18) whereas others have found a null or even protective effect of wine consumption on breast cancer risk.(6,19,20) This effect has been attributed to polyphenols in wine, and particularly resveratrol, a phytoalexin produced by grape vines in response to injury and found in red wine.(21-23) In vitro, resveratrol inhibits tumor initiation and progression by inducing cell cycle arrest and apoptosis.(22,24,25) One pathway by which this occurs is mediated by *BRCA1*. As a phytoestrogen, resveratrol binds to the estrogen receptor and up-regulates transcription of *BRCA1* and *BRCA1*-associated proteins in human breast cancer cell

lines.(25,26) In mouse models, resveratrol is a potent inhibitor of the initiation and progression of *BRCA1* mutant cancer, suggesting that resveratrol may be a potential chemopreventive agent for women with *BRCA1* mutations.(24)

In a previous study among *BRCA1* and *BRCA2* mutation carriers less than 50 years of age from the United States, Canada, and Australia, no increase in the risk of breast cancer was seen in relation to increasing alcohol consumption.(27) In a small study, our group also previously reported no association between alcohol consumption and breast cancer risk, regardless of alcohol type, among 137 French-Canadian *BRCA1* and *BRCA2* mutation carriers, some of whom were included in the present analysis.(28) Although alcohol type was considered in these studies, they may have been underpowered to detect an association between breast cancer risk and wine consumption, due to the low prevalence of wine consumers.

The major strength of this study is the large sample size of women with known *BRCA* mutations. It provides one of the rare assessments of the association between alcohol consumption and breast cancer risk among women with *BRCA* gene mutations. However, our study has several limitations, including the possibility of recall bias and under-reporting of alcohol consumption. Participants in this study reported current alcohol consumption patterns, which may not reflect exposure prior to diagnosis. We did not have information on alcohol consumption patterns earlier in life. However, recent prospective, population-based evidence suggests that alcohol consumption patterns do not change appreciably following breast cancer diagnosis.(29) Furthermore, if the present results were driven by a reduction in alcohol consumption following breast cancer diagnosis, we would expect to see a protective effect among *BRCA2* mutation carriers as well. Using non-drinkers as the referent group may have induced a spurious association between alcohol consumption and breast cancer risk, as non-

drinkers may differ systematically from the rest of the population in other behaviours associated with breast cancer risk. Our analysis restricted to drinkers only did not find an association between alcohol consumption and breast cancer risk among *BRCA1* mutation carriers, but may have been underpowered to detect an effect. This study is also limited by the crude measures of alcohol consumption, and no information was available on red versus white wine consumption. The study population was young and predominantly premenopausal; this may influence the generalizability of the results to older *BRCA1* and *BRCA2* carriers.

This study may be susceptible to survivor bias if breast cancer patients who consumed alcohol experienced reduced survival, compared to non-drinkers. To assess this possibility, we restricted analyses to the 794 *BRCA1* and 247 *BRCA2* pairs in which the case had completed the questionnaire within five years of breast cancer diagnosis. The odds ratio associated with current alcohol consumption was 0.73 (95% CI 0.58-0.92) for *BRCA1* carriers and 0.87 (95% CI 0.54-1.34) for *BRCA2* carriers, similar to results for the entire study population. Findings for the number of alcoholic drinks consumed per week and the type of alcohol regularly consumed among recently diagnosed cases were similar to those for the full study population (Appendices L and M).

In conclusion, we found that increasing consumption of wine was associated with a reduced risk of breast cancer among women with a *BRCA1* mutation, while alcohol consumption has no effect among women with a *BRCA2* mutation. The possible protective effect of wine found in this study is in accordance with some, but not all, studies of women in the general population and provides support for the potential role of resveratrol in *BRCA1*-associated breast cancer chemoprevention. Further studies in this area are needed to confirm this apparent protective effect of wine consumption against breast cancer among women with a *BRCA1*

mutation. Future studies should include large numbers of *BRCA* mutation carriers and should distinguish between red and white wine consumption

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5. Discussion and Conclusions

5.1. Summary of Principal Findings

This thesis examined how the relationship between alcohol consumption and breast cancer risk was modified by mutations in the *BRCA1* and *BRCA2* genes using a case-only and a case-control design. In Chapter 2, it was shown that the case-only study design was a valid and useful method of measuring departure from multiplicative gene-gene and gene-environment interactions. Potential sources of bias existed, including violations of the independence assumption. However, the mean IOR_{CC}/IOR_{CO} ratio calculated from 24 empirical evaluations found no significant difference from unity (IOR_{CC}/IOR_{CO} ratio=1.06, 95% CI, 0.93-1.22), suggesting that both the case-only and case-control methods yielded equivalent estimates of interaction. The small amount of between-study variation in the IOR_{CC}/IOR_{CO} ratio (I^2=23.9%) was not explained by any study methodological features.

In Chapter 3, the application of the case-only method to a sample of French-Canadian breast cancer patients to measure interactions between *BRCA1* and *BRCA2* gene mutations and alcohol consumption was reported. Out of 857 women included in the study, 10 carried a mutation in *BRCA1* and 33 carried a mutation in *BRCA2*. The interaction between total wine consumption and *BRCA1* gene mutations tended towards a less than multiplicative effect (IOR_{CO}=0.38, 95% CI 0.08-1.81) whereas the interaction between total beer, fortified wine, and spirit consumption and *BRCA1* did not (IOR_{CO}=2.49, 95% CI 0.64-9.73). The joint effect of mutations in the *BRCA2* gene and total beer, fortified wine, and spirit consumption was significantly greater than the product of the independent effects of each (IOR_{CO}=2.15, 95% CI 1.03-4.49).

The findings reported in Chapter 3 were partially supported by those in Chapter 4. In a matched case-control study of 1480 pairs of *BRCA1* mutation carriers and 445 pairs of *BRCA2* mutation carriers, increasing current consumption of alcohol was associated with a reduced breast cancer risk among *BRCA1* mutation carriers (p=0.03) but not *BRCA2* mutation carriers (p=0.72). Among the 895 *BRCA1* mutation carrier pairs in which the type of alcohol consumed was known, increasing exclusive consumption of wine was associated with a reduced risk of breast cancer (p-trend=0.01). The trends for increasing non-exclusive wine consumption were not significant. There was no association between the type of alcohol consumed and breast cancer risk among the 246 *BRCA2* mutation carrier pairs in which the type of alcohol consumed was known.

5.2. Interpretation and Comparison with Other Related Studies in the Literature

Gene-environment and gene-gene interactions lie at the root of many human diseases.(1-3) Studying these interactions can provide insight on the causes, pathogenesis, and prevention of disease, for example by elucidating disease pathways or by identifying population subgroups that are genetically susceptible to certain exposures.(4-6) As more genes are discovered and characterized in the post Human Genome Project era, the number of investigations of gene-environment and gene-gene interactions will also increase. In 2000, 136 studies (21.0% of total) in the Human Genome Epidemiology (HuGE) literature database reported on genetic interactions, compared to 2089 studies (26.6% of total) in 2009.(7) For these reasons, it is important to have efficient and reliable epidemiological study designs to measure interactions.

Traditionally, case-control studies, and case-control studies nested within a prospective cohort study, are used to measure gene-environment and gene-gene

interactions.(5,6) The advantages of the stand-alone case-control design are that large samples can be ascertained and detailed exposure and disease phenotype information can be collected. However, the design may be subject to selection bias, as it is often difficult to find a control group representative of the population in which cases occurred; biologic specimens may be difficult to obtain, especially from controls, due to cost and ethical considerations; exposure recall may differ between cases and controls; and population stratification could confound the results.(5,8) Nested case-control studies have a lower potential for selection and information bias, but are inefficient if the disease is rare and must be sampled from a cohort in which high follow-up rates have been maintained. More importantly, to detect a departure from multiplicative effects, both study designs require sample sizes approximately four times greater than those required to detect main effects.(5) In the presence of measurement error, even larger sample sizes are required.(9,10)

By removing the need for a control group, the case-only design avoids these problems. For example, to detect an interaction effect of 6.1 between maternal cigarette smoking and transforming growth factor alpha (TGFA) *Taq1* polymorphism on the risk of cleft palate in a population based sample of infants with birth defects with 80% power (assuming exposure prevalence of 0.25, susceptibility genotype prevalence of 0.16, and marginal exposure and genotype risks of 1 and 0.9 respectively), 375 subjects would be needed in a case-control study, compared to 55 in a case-only study.(11) Similarly, under a range of genotype frequencies and relative risks, the case-only design was at least 2.44 times as efficient as the case-control design in detecting a gene-gene interaction of 3.0 with 80% power.(12) The systematic review showed that in addition to these gains in

efficiency, the case-only design gives estimates of interaction comparable to those obtained from other study designs.

The systematic review was not able to address the question of measuring and interpreting interaction, which is often debated in epidemiology.(13-15) Statistical interaction, also known as heterogeneity of effect, refers to the need to include an interaction, or product, term in a statistical model in order for the model to fit the data well.(14) Interaction is measured on either a multiplicative or additive scale, depending on whether the outcome is a risk ratio or risk difference. Statistical interaction is easy to implement, but has been criticized for being arbitrary and not reflective of the underlying biological processes.(4) Biologic interaction on the other hand, refers to the joint effect of two factors in contributing to disease development and follows an additive interaction model.(4,14) The case-only design measures departures from a multiplicative model of interaction. Nonetheless, departures from the multiplicative model are biologically plausible for many gene-environment interactions.(16)

The systematic review also emphasized the importance of complete and transparent reporting of study methods and results. Incomplete reporting hampers evaluation of potential sources of bias, interpretation of results, and incorporation of studies into systematic reviews.(17,18) The issue has been raised in the fields of clinical trials (19), systematic reviews (20), and observational epidemiology.(21) To combat the problem, committees of experts have developed reporting guidelines in their respective fields. Examples are the CONSORT (Consolidated Standards of Reporting Trials, published in 1996 and updated in 2001) (22), PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses, originally published as the QUOROM statement

in 1999 and updated in 2007) (23), and STROBE (Strengthening the Reporting of Observational Studies in Epidemiology, published in 2007) (24) statements. Improvements in reporting consequent to the adoption of the CONSORT statement have been found.(25)

Transparent reporting of HuGE studies is of special concern for several reasons. First, associations found in HuGE studies tend to be small.(26) Second, many gene-disease associations are not replicated in subsequent research. A literature review in 2002 identified 603 gene-disease associations, of which 166 were assessed in three or more separate publications.(27) Of these, only 6 associations were consistently replicated. A meta-analysis of 36 gene-disease associations published in 370 studies found that the odds ratio from the first study was only modestly correlated with odds ratios found in subsequent studies.(28) This lack of replication may be due to the large number of genes and outcomes studied, increasing the potential for spurious associations and selective reporting of positive associations, or it could be due to differences in study design.(18,29)

The problems of small magnitude of effect and lack of replication in HuGE studies are best addressed by synthesizing and integrating study results, which requires transparent reporting. The STREGA (Strengthening the Reporting of Genetic Association Studies) statement (30), an extension of the STROBE statement and published in 2009, has been developed to help this process. The STREGA statement outlines additions to items on the STROBE checklist that are relevant to genetic association studies, such as genotyping errors, Hardy-Weinberg Equilibrium (HWE), selection of participants, relatedness of subjects, and volume of data issues. These items

served to guide the selection of study design parameters evaluated in the meta-regression analysis. The meta-regression analysis found that none of the design parameter explained any of the between-study heterogeneity in the IOR_{CC}/IOR_{CO} ratio. However many of the design parameters included were incompletely reported (e.g. HWE, selection of controls, participation rate). This underscores the need for adherence to the STREGA statement in HuGE studies so that evidence on the influence of study characteristics can accumulate.

Interactions between breast cancer risk factors and mutations in the *BRCA* genes are rarely assessed in population-based studies of breast cancer due to the low population prevalence of these mutations.(31) Instead, collaborations of researchers have pooled their databases of affected and unaffected mutation carriers. In addition to the Hereditary Breast Cancer Clinical Study Group headed by Dr. Narod in Toronto, there is the International *BRCA1*/2 Carrier Cohort Study (IBCCS); the PROSE and MAGIC consortia involving approximately 24 centres in Europe and North America; and the group led by the Breast Cancer Family Registry (BCFR) that uses data from the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (KConFab) and the Ontario Cancer Genetics Network (OCGN).(32) Through these collaborations, some of the breast cancer risk factors that have been identified among women in the general population have also been found among women with *BRCA* mutations, suggesting no interaction between these risk factors and mutations in the *BRCA1* or *BRCA2* genes.(32,33)

A notable exception is alcohol consumption. Most studies on women in the general population have found that alcohol consumption increases breast cancer risk, regardless of the type of alcohol consumed.(34-37) An early study by the Narod group of

89 affected and 48 unaffected *BRCA* mutation carriers (38) found no association between alcohol consumption and breast cancer risk, regardless of the type of alcohol consumed, although *BRCA1* and *BRCA2* mutation carriers were not considered separately. In a study of mutation carriers diagnosed before 50 years of age, the BCFR group reported no association between alcohol consumption and breast cancer risk among 195 affected and 302 unaffected *BRCA1* mutation carriers.(39) An inverse association with modest alcohol consumption (1-4 g/day) compared to no alcohol consumption was observed among 128 affected and 179 unaffected *BRCA2* mutation carriers.

In light of the paucity of evidence on breast cancer risk and alcohol consumption among *BRCA* mutation carriers, this thesis sought to address the question using two different study designs: a case-only study of a sample of French-Canadian breast cancer patients, and a case-control study of a large sample of affected and unaffected *BRCA* mutation carriers. The case-control analysis in Chapter 4 found that increasing wine consumption had an inverse association with breast cancer risk among *BRCA1* mutation carriers, which supported the finding from the case-only analysis. However the case-control analysis found no association between alcohol consumption and breast cancer risk among *BRCA2* mutation carriers, which was not in accordance with the case-only analysis.

Alcohol consumption patterns vary considerably both between and within countries and depend on social acceptability, cost, and accessibility.(40,41) Globally, it has been estimated that alcoholic drinks contribute an average of 2.3% of total dietary energy.(40) Women consume less alcohol than men.(42) They also tend to consume less

beer and, to a lesser extent, spirits, as they age, but wine consumption remains relatively constant throughout adult life (42,43) and may even increase with age.(44)

Alcohol consumption patterns also vary with markers of socioeconomic status (SES). In studies among the United States and Danish populations, wine consumption increased with increasing educational attainment, IQ, and social status, while beer consumption was inversely associated with educational attainment, IQ, and social status.(42,45) Higher SES is often associated with other health-promoting behaviours and has been hypothesized to explain "The French Paradox", in which red wine consumption was associated with lower rates of atherosclerosis-related deaths, despite diets high in fat.(46) However, breast cancer incidence rates increase with higher SES.(47) It is therefore unlikely that the observed association between wine consumption and reduced breast cancer risk among *BRCA1* mutation carriers is due to confounding by SES.

Alcohol consumption and smoking are highly correlated with one another.(48) If smoking increases breast cancer risk, then the association between alcohol consumption and breast cancer could be confounded by smoking. The pooled re-analysis of 53 studies by the Collaborative Group on Hormonal Factors in Breast Cancer found that the relationship between alcohol consumption and breast cancer was not confounded by ever or never smoking.(48) However, they did not consider pack-years of smoking. The relationship between smoking and breast cancer risk is controversial. A Canadian Expert Panel was convened in 2008 to examine the issue and concluded that early age at smoking initiation, increasing pack-years of smoking, and longer duration of smoking increased breast cancer risk, regardless of alcohol consumption.(49) They also concluded

that secondhand smoke exposure increased the risk of breast cancer in younger, primarily premenopausal women who had never smoked.

In addition, the panel found evidence for an increase in breast cancer risk among genetically susceptible women, including those with *BRCA* gene mutations.(49) A BCFR consortium study found that five or more pack-years of smoking increased the risk of breast cancer more than two-fold among *BRCA1* and *BRCA2* mutation carriers.(50) The authors of this study discussed how this was biologically plausible since smoking increases the likelihood of double strand breaks (DSB), which *BRCA* mutation carriers have an impaired ability to repair. A study by the Narod group found a slightly increased risk of breast cancer associated with increasing pack-years of smoking among *BRCA1* mutation carriers who were past, but not current smokers.(51)

In support of these findings, in the present thesis, there was some evidence of higher breast cancer risks associated with alcohol consumption among smokers in both the case-control and case-only analyses. In the case-control analysis, among *BRCA1* mutation carriers who had never smoked, the trend for increasing alcohol consumption was inversely related to breast cancer risk (p-trend=0.01) whereas among those who had ever smoked, the trend was not significant (p-trend=0.37). In the case-only analysis, the more than multiplicative effect of alcohol consumption and *BRCA2* gene mutations was only present in ever smokers (IOR_{CO}=2.28, 95% CI 0.94-5.55) but not in never smokers (IOR_{CO}=0.92, 95% CI 0.22-3.92), although the difference was not statistically significant. Because we used a crude measure of smoking (ever/never) in our analyses, residual confounding by smoking may have biased the results.

Differences in the characteristics of subjects included in the case-only and case-control analyses could in part explain the discrepancy in the findings for *BRCA2* mutation carriers. Compared to the subjects in the case-control analysis, the patients in the case-only analysis were older at diagnosis and were less likely to be premenopausal. As described in Chapter 4, the pooled analysis of 53 studies by the Collaborative Group on Hormonal Factors in Breast Cancer found a slightly higher increase in breast cancer risk per 10 g/day alcohol consumed among postmenopausal (8.1%, 95% CI 5.6-10.6%) compared to premenopausal (6.3%, 95% CI 3.6-9.0%) women.(48) Therefore, the higher risks among *BRCA2* carriers in the case-only compared to the case-control analysis may be explained by the higher prevalence of postmenopausal women. Alternatively, the higher prevalence of other breast cancer risk factors such as smoking, oral contraceptive use, and HRT use in the case-only subjects, and the lack of adjustment for these factors in the analysis, may have confounded the case-only results.

In the case-only study, information about alcohol consumption patterns was assessed by a food frequency questionnaire in which the type and amount of alcohol were clearly defined. In contrast, participants in the case-control study specified the number of drinks they consumed per week on average (0-3, 4-9, 10-20, or 20 or more) and the type of alcohol regularly consumed. Differences in the definition of a 'drink' from one country to another (e.g. one US 'drink' is approximately 15 g ethanol, whereas a 'drink' in the UK is 8 g ethanol) (40), as well as the crude measure of alcohol consumption, increased the possibility of misclassification. Assuming this was non-differential by case-control status, this would have biased the results of the case-control study towards the null. When comparing the case-only and case-control results, it should also be noted

that alcohol consumption was assessed in the year before diagnosis in the case-only study, and current alcohol consumption was assessed in the case-control study. Although there is empirical evidence to support no change in alcohol consumption patterns following breast cancer diagnosis (52), this potential source of bias should not be dismissed entirely and would also have biased the results of the case-control analysis towards the null.

Chance may also explain the different results for *BRCA2* mutation carriers in the case-only and case-control analyses. The significant more than multiplicative interaction seen in the case-only analysis may be spurious (type I error). Alternatively, the case-control analysis may have been under-powered to detect an effect (type II error). In support of the latter, breast cancer risk increased non-significantly as alcohol consumption increased among *BRCA2* mutation carriers in the case-control study.

5.3. Strengths and Limitations of the Thesis

The main strength of this thesis is the flow of information from one chapter to the next. Chapter 2 concludes that the case-only study is a valid and powerful design for measuring gene-environment interactions. In Chapter 3, this design is applied to measure the interaction between *BRCA* gene mutations and alcohol consumption in a real dataset. Also, a method to control for bias in the case-only design, namely, adjusting for the source of non-independence, was identified in the systematic review and used in the real data example. The results from Chapter 4 in part support the findings from the case-only analysis and reasons for the discrepancy are explored in Chapter 5.

A number of limitations of this thesis also warrant consideration. First, since *BRCA* mutation carriers identified at CHUM, Hôtel-Dieu also contribute data to the

Hereditary Breast Cancer Clinical Study Group, some of the cases with *BRCA* mutations included in the case-only analysis may also have been included in the case-control analysis. Nonetheless, the 1925 cases in the case-control analysis were selected from a larger sample of 2707 cases, so that not all CHUM cases would necessarily have been included. Removal of the French-Canadian pairs (representing 3 centres) from the case-control analysis did not change the case-control results, suggesting that the results were not driven by the French-Canadian patients.

Second, in both the case-only and case-control studies, family members of affected mutation carriers were invited to participate. Familial clustering of breast cancer risk factors could have inflated the standard errors of parameter estimates, increasing the rate of false positives.(53) To adjust for familial clustering, other studies of *BRCA* mutation carriers have used robust variance estimators to measure the standard errors.(54) Neither the case-only nor the case-control study took the relatedness of included subjects into consideration and therefore the possibility of type I errors cannot be excluded.

Third, both study designs used at least some prevalent cases, which required cases to survive long enough to be included in the study. If alcohol consumption is associated with a reduced risk of survival among breast cancer patients, this could result in prevalence/incidence (Neyman) bias, a type of survival bias.(55) Effect estimates would be biased towards the null, or this could result in an artificial inverse association, such as was seen for *BRCA1*. However, as discussed in Chapters 3 and 4, restricting the analyses to recently diagnosed cases did not significantly change the results in either study. Furthermore, if Neyman bias was an explanation for the results, we would have expected to see an inverse association with alcohol consumption among *BRCA2* mutation carriers

as well. Lastly, there is little empirical evidence that alcohol consumption affects breast cancer survival. Among 1282 women with invasive breast cancer and followed for an average of over 13 years in the Nurses' Health Study, alcohol consumption following breast cancer diagnosis was not associated with survival.(56) Similar results for ever and current drinking were observed among 1,453 Italian breast cancer patients followed for a median of 12.6 years.(57)

Finally, participants in both study designs were sampled from high-risk families or were selected because they were likely to have a hereditary basis for the disease. It is possible that other genetic or environmental factors for breast cancer may also cluster in these families and the results of the current analyses may not be applicable to the general population of *BRCA* mutation carriers.

5.4. Unanswered Questions and Future Research

This thesis provides one of the rare assessments of alcohol consumption and breast cancer risk in relation to *BRCA* gene mutations. It is the first to report a potentially protective effect of wine consumption among *BRCA1* mutation carriers and it provides unclear results about the risks to *BRCA2* mutation carriers. Selection bias, information bias, confounding, and chance cannot be completely ruled out as explanations for the results and these findings should be confirmed in other studies. The most robust estimates of cancer risks to mutation carriers will come from prospective cohort studies. This type of analysis is only now becoming possible as enough unaffected cohort members are being followed for sufficient amounts of time.

The study of genetic risk modifiers of *BRCA1*- and *BRCA2*-associated breast cancer also holds potential. Identification of other susceptibility genes could contribute

to our understanding of hereditary breast cancer and may be applicable to women in the general population as well.(32) Recognizing this need, the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) (58) was established in 2005 with a goal to amass sufficient DNA samples from *BRCA* mutation carriers to make this possible. Since then, the consortium has amassed over 10,000 samples and has investigated associations between breast cancer risk and polymorphisms in genes whose proteins act in the same pathway as the *BRCA* proteins (59,60), as well as polymorphisms found to be associated with breast cancer risk in the general population.(61) The case-only design could be used to confirm findings from these studies and to explore new gene-gene interactions with *BRCA* mutations and should be considered in future research.

5.5. Conclusions

In conclusion, this thesis found that the case-only study was a valid design to measure departures from multiplicative gene-gene and gene-environment interactions. The design may be especially useful when the disease is truly rare or when information on cases is readily available. A case-control analysis of *BRCA* mutation carriers found that wine consumption may reduce breast cancer risk among *BRCA1* mutation carriers, and the results of case-only analysis may be compatible with this. The effects of alcohol consumption on breast cancer risk among *BRCA2* mutation carriers are unclear. These findings should be confirmed in larger studies and preferably in prospective cohort studies.

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Appendix A

The Case-Only Study Design

The derivation of the case-only interaction estimate, and its relation to the cohort and case-control interaction estimates, is described below. In all study designs, interaction is measured on a multiplicative scale as the extent to which the joint effect of genotype and environment (or of genotype and genotype in studies of gene-gene interaction) differs from the product of the independent effect of each.

The source population can be presented in the following 2x4 table:

Environment (e)	Genotype (g)	Disease	No disease	Total	Relative Risk (RR)
-	-	a	B	a+B	1.0 (ref)
-	+	c	D	c+D	$RR_g = \frac{c(a+B)}{a(c+D)}$
+	-	e	F	e+F	$RR_e = \frac{e(a+B)}{a(e+F)}$
+	+	g	H	g+H	$RR_{ge} = \frac{g(a+B)}{a(g+H)}$

One can get the gene-environment interaction relative risk (*IRR*) based on the cohort data:

$$IRR_{true} = \frac{RR_{ge}}{RR_g RR_e} = \left(\frac{ag}{ce}\right)\left(\frac{(c+D)(e+F)}{(a+B)(g+H)}\right) = (IOR_{CO})\left(\frac{(c+D)(e+F)}{(a+B)(g+H)}\right) \quad \textbf{(1)}$$

Where IOR_{co} is the interaction odds ratio based on cases only.

To generate case-control data from the above source population, we use density control sampling and take a 100% sample of the people with disease and 'p' of the non diseased people. This gives us the following 2x4 table (after cancelling out the 'p's):

Environment (e)	Genotype (g)	Case	Control	Odds Ratio (OR)
-	-	a	b = pB	1.0 (ref)
-	+	c	d = pD	$OR_g = \frac{cb}{ad} = \frac{cB}{aD}$
+	-	e	f = pF	$OR_e = \frac{eb}{af} = \frac{eB}{aF}$

+	+	g	h = pH	$OR_g = \frac{gb}{ah} = \frac{gB}{aH}$

One can get the gene-environment interaction odds ratio (*IOR*) based on the case-control data:

$$IOR_{cc} = \frac{OR_{ge}}{OR_g OR_e} = \left(\frac{ag}{ce}\right)\left(\frac{DF}{BH}\right) = (IOR_{co})\left(\frac{DF}{BH}\right) \quad (2)$$

Where IOR_{co} is the interaction odds ratio based on cases only.

To generate case-only data from the above source population, we use density control sampling as above and consider only the 'case' column. This gives us the following 1x4 table (after cancelling out the 'p's):

Environment (e)	Genotype (g)	Case
-	-	a
-	+	c
+	-	e
+	+	g

One can get the gene-environment interaction odds ratio (*IOR*) based on the case-only data:

$$IOR_{co} = \left(\frac{ag}{ce}\right) \quad (3)$$

Relationships

From equation (1), the requirement for equality of the cohort and case-only interaction estimates is:

$$\left(\frac{(c-D)(e-F)}{(a-B)(g-H)}\right) = 1$$

This means that the environmental factor and the genetic factor must be **independent in the entire source population**. This assumption must be assessed directly in the source population. Alternatively, if this assumption is evaluated only among a group of people without the disease, the disease must be rare in the population in order to conclude that the case-only interaction is an unbiased estimate of the cohort interaction effect. Note, however, that the equality of the cohort and case-only interaction estimates does not require a rare disease assumption.

From equation (2), the requirement for equality of the case-control and case-only interaction estimates is:

$$\left(\frac{DF}{BH}\right) = \frac{df}{bh} = 1$$

This means that the environmental factor and the genetic factor must be **independent in people without the disease**. This can be assessed in the control group or in the base population. Note that a rare disease assumption is not required for the case-only and case-control interaction estimates to be equivalent. Naturally, even if the interaction odds ratios are the same, this does not mean that either is unbiased relative to the true risk ratio.

From equations (1) and (2), the requirement for equality of the case-control and cohort interaction estimates is:

$$IOR_{cc} = IRR_{true}\frac{(DF)}{(BH)}\left(\frac{(a+B)(g+H)}{(c+D)(e+F)}\right)$$

or, equivalently,

$$\frac{(DF)}{(BH)}\left(\frac{(a+B)(g+H)}{(c+D)(e+F)}\right) = 1$$

This means that genetic and environmental factors must be **independent in people without the disease and independent in the source population**. The case-control and cohort interaction estimates will also be equivalent if the disease is rare, even if the genetic and environmental factors are not completely independent in either the population or among people without disease, because the term

$$\frac{(DF)}{(BH)}\left(\frac{(a+B)(g+H)}{(c+D)(e+F)}\right)$$

will be close to 1. The assumption of a rare disease can only be assessed in the entire source population.

Appendix B

Search strategy used in systematic review. Last executed October 7, 2009.

EMBASE, MEDLINE	PUBMED	CINAHL	Clinicaltrials.gov[a]
1. (case adj case).tw	1. "case case" [Text Word]	("case only" OR "case case") AND (genetics/ OR gene* OR heredit* OR inherit* OR genotyp*) Limit 1994-2009	case-only AND (gene OR genetic OR heredity OR inherit OR inheritance OR genotype OR genotyped OR genotypes OR genotypic)
2. (case adj only).tw	2. "case only" [Text Word]		
3. 1 or 2	3. #1 OR #2		
4. genetics/	4. "genetics"[MeSH Terms]		
5. gene$.tw	5. gene* [Text Word]		
6. heredit$.tw	6. heredit* [Text Word]		
7. inherit$.tw	7. inherit* [Text Word]		
8. genotyp$.tw	8. genotyp* [Text Word]		
9. or/4-8	9. #4 OR #5 OR #6 OR #7 OR #8		
10. 3 and 9	10. #3 AND #9		
11. limit 10 to English	11. Limits: Publication Date from 1994/01/01 to 2009/10/7, English		
12. limit 11 to yr="1994 - Current"			

[a]Last executed on November 9, 2009

Appendix C

Qualitative comments extracted from included studies.

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
Albert, P. S., D. Ratnasinghe, J. Tangrea, and S. Wacholder. 2001. Limitations of the case-only design for identifying gene-environment interactions. American Journal of Epidemiology 154, (8) (Oct 15): 687-93.	Theoretical evaluation; Empirical comparison of case-only, case-control, and cohort designs using simulated data; Empirical comparison of case-only and case-control designs using real data	"Although the assumption of independence for a gene and an external environmental factor or behavior seems reasonable, there will rarely be sufficient empirical data to support the independence assumption." "The case-only design is very sensitive to the independence assumption. One either has to have a great deal of confidence in the independence assumption or evaluate it empirically with data on controls." "Evidence of the gene-environment association [in controls] could be used directly or in a sensitivity analysis to correct for a sizable gene-environment association by scaling the case-only estimate by the reciprocal of the gene-		"[In the real data example] the corresponding case-only estimates are 0.90 and 0.47, indicating that the inferences for assessing gene-environment interactions may be very different for the case-control and case-only designs." "For a log-odds ratio assessing independence larger than 0.2 (odds ratio of only 1.22), estimates of the mean squared error (which penalizes for bias and variance) are larger for the case-only than the case-control design."	"The case-only design is subject to methodological problems such as uncontrolled confounding, exposure misclassification [that is differential by mutational status], and nonresponse bias."

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		environment association or to support the independence assumption if the gene-environment odds ratio is near 1." "Relying on tests of independence in the controls is not effective because of their generally low power to detect meaningful departures from independence." "The association between an environmental factor and a genetic marker in the controls could be due to genes associated with or causal for behaviors such as smoking and alcohol drinking." "Polymorphisms can be associated with behavior through linkage equilibrium. Furthermore, an association may be induced by ignoring an important stratification variable (i.e., uncontrolled confounding) that relates to both factors." "Alternatively, dependence could possibly be induced by differential participation in the study, even if the factors are independent in the			

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		population."			
Bai, Y., L. R. Goldin, and A. M. Goldstein. 2001. Gene x environment interaction from case-control and case-case approaches. *Genetic Epidemiology* 21, (Suppl 1): S825-30.	Empirical comparison of case-only and case-control designs using simulated data	"The cross-product ratio depends on both the interaction and population association of gene 1 and sex. If these two factors are independent in the population, the cross-product ratio is equal to a risk ratio."		"We found a similar but significant result [in the case-case analysis] compared with the one in the case-control study.	
Becher, H., S. Schmidt, and J. Chang-Claude. 2003. Reproductive factors and familial predisposition for breast cancer by age 50 years. A case-control-family study for assessing main effects and possible gene-environment interaction. *International Journal of Epidemiology* 32, (1) (Feb): 38-48.	Empirical comparison of case-only and case-control designs using real data	"If the factors are not independent, the case-only analysis is not appropriate."		"The interaction estimates [for genetic susceptibility and breastfeeding] are close to one with large p-values." "The interaction estimates [for genetic susceptibility and abortion] are mostly positive but not statistically significant." "We found consistent negative interaction estimates in all models and analyses considered, indicating a stronger protective effect of later age at menarche for women with genetic susceptibility."	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
Caceres, D. D., J. Iturrieta, C. Acevedo, C. Huidobro, N. Varela, and L. Quinones. 2005. Relationship among metabolizing genes, smoking and alcohol used as modifier factors on prostate cancer risk: Exploring some gene-gene and gene-environment interactions. European Journal of Epidemiology 20, (1): 79-88.	Empirical comparison of case-only and case-control designs using real data			"When we assessed gene-gene through SIM, more than multiplicative interactions were observed within GSTM1-GSTT1 and GSTT1-CYP1A1 susceptibility genotypes in both epidemiologic designs [case-only and case-control] indicating a modifier effect of these genes on PCa risk."	
Caceres DD, Quinones LA, Schroeder JC, Gil LD, Irarrazabal CE (2009) Association between p53 codon 72 genetic polymorphism and tobacco use and lung cancer risk. Lung 187: 110-115.	Empirical comparison of case-only and case-control designs using real data			"Both approached (case-control and case-only) revealed a significant synergistic effect between these factors compared with each risk factor alone"	
Chang-Claude, J., A. Dunning, U. Schnitzbauer, P. Galmbacher, L. Tee, M. Wjst, J. Chalmers, et al. 2003. The patched polymorphism Pro1315Leu (C3944T) may modulate the association between use of oral	Empirical comparison of case-only and case-control designs using real data			"In the DKFZ population-based case-control study, we observed a significant interaction between 1315Leu-carrier status and use of oral contraceptives. This	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
contraceptives and breast cancer risk. *International Journal of Cancer* 103, (6) (Mar 1): 779-83.				was confirmed in case-only analysis of the DKFZ study." "There was no evidence of heterogeneity between the results of the two studies."	
Chen Y-, Lin H-, Liu H (2009) Two-stage analysis for gene-environment interaction utilizing both case-only and family-based analysis. Genet Epidemiol 33: 95-104.	Theoretical evaluation; Empirical comparison of case-only and case-family designs using simulated data	"Although the G-E independence assumption is believed to be reasonable in general since the genetic factors are determined at birth while the environmental factors are acquired through life, there still remain plausible settings where such an assumption fails." "Owing to population stratification, even though there is perfect G-E independence in each stratum, there could exist overall correlation between genotypes and environmental exposures in the study population that eventually invalidates a case-only analysis." "Another important reason for the presence of genotype-exposure dependencies is the		"Family-based analysis usually requires weaker assumptions on distributions of genetic and environmental factors than case-only analysis." "Where population stratification exists… the family-based estimator is essentially unbiased. On the contrary, the case-only estimator is biased for B_{ge}" "The case-only design… is vulnerable to bias due to gene-environment dependencies in the study population, while the case-parent/ case-sibling design is	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		confounding of family history, which is apparently associated with both susceptibility genes and lifestyle exposures."		more robust to such bias."	
Cheng, K. F., and J. H. Chen. 2005. Bayesian models for population-based case-control studies when the population is in hardy-weinberg equilibrium. Genetic Epidemiology 28, (2) (Feb): 183-92.	Theoretical evaluation; Empirical comparison of case-only and case-control designs using real data			"Basically, the previous conclusions for [the interaction parameters] are unchanged [using case data only compared to case and control data]."	
Cheng, K.F. 2006. A maximum likelihood method for studying gene-environment interactions under conditional independence of genotype and exposure. Statistics in Medicine 25, (18) (Sep 30): 3093-109.	Theoretical evaluation; Empirical comparison of case-only and case-control designs using simulated data	"If there exists no interaction between genetic factor G and factor W [another binary risk factor], the usual cross-product ratio still estimates GxE interaction. Otherwise, some kind of adjustment needs to be made in order to eliminate the bias of estimation. A correct way of removing the bias is to control for the covariate W in the logistic regression analysis, provided G and (E,W) are independent or G and E are conditionally independent given W in the control population." "We have provided a			

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		methodological guideline for what types of additional variables should be included in case-only analyses in order to remove the bias caused by non-independence between G and E or some other reasons. "			
Cheng, K. F. 2007. Analysis of case-only studies accounting for genotyping error. *Annals of Human Genetics* 71, (Pt 2) (Mar): 238-48.	Theoretical evaluation				"Under the null hypothesis [of no GxE interaction], the LR test is not affected by genotyping errors... The biases and variances of the ML estimates of the GxE interactions also remain rather stable and small in the null case as the value of the error rate varies. However, under the alternative,... we have more bias concerning the values of the true parameters." "A simulation shown in this paper reveals that ignoring the presence of

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
					genotyping errors may seriously increase the bias of the point estimate."
Cheng KF, Lin WJ (2009) The effects of misclassification in studies of gene-environment interactions. Hum Hered 67: 77-87.	Theoretical evaluation				"Two major sources of bias in case-control or case-only studies are population stratification and misclassification of genotype and /or environmental exposure." "The results indicate that the level of the bias, due to misclassification, depends on misclassification probabilities and joint frequencies of G and E across strata." "We consider a general model that misclassification of genotype and exposure are conditionally independent. Based on this model, we show that the effect

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
					of misclassification is nonexistent in case-control or case-only studies when (G,S)-E independence or G-E conditional independence holds in the control population (where S denotes a general stratification variable)."
Clavel, J., S. Bellec, S. Rebouissou, F. Menegaux, J. Feunteun, C. Bonaiti-Pellie, A. Baruchel, et al. 2005. Childhood leukaemia, polymorphisms of metabolism enzyme genes, and interactions with maternal tobacco, coffee and alcohol consumption during pregnancy. European Journal of Cancer Prevention 14, (6) (Dec): 531-40.	Empirical comparison of case-only and case-control designs using real data			"The OR associated with the interaction term estimated from the case-control approach was close to that from the case-only approach for CYP1A1, but not at all for GSTM1."	
Deng, Y., B. Newman, M. P. Dunne, P. A. Silburn, and G. D. Mellick. 2004. Case-only study of interactions between genetic polymorphisms of GSTM1, P1, T1 and Z1 and smoking in parkinson's disease. Neuroscience Letters 366, (3)	Empirical comparison of case-only and case-control designs using real data			"The corresponding ORi case-control values were of similar magnitude and in the same direction to those obtained using the case-only design."	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
(Aug 19): 326-31.					
Dowling, N. F., H. Austin, A. Dilley, C. Whitsett, B. L. Evatt, and W. C. Hooper. 2003. The epidemiology of venous thromboembolism in caucasians and african-americans: The GATE study. *Journal of Thrombosis & Haemostasis* 1, (1) (Jan): 80-7.	Empirical comparison of case-only and case-control designs using real data	"Case-only analysis requires that the exposures are statistically independent."	"With respect to the evaluation of the interaction odds ratio, the case-only method substitutes the need for a control group with the assumption that the two exposures are independent. For genetic traits, this assumption can be evaluated easily by consideration of linkage disequilibrium, so that genetic studies are good candidates for case-only analyses."	"[The case-control analysis] supports the findings of the case-only analysis."	
Egan, K. M., P. A. Newcomb, L. Titus-Ernstoff, A. Trentham-Dietz, L. I. Mignone, F. Farin, and D. J. Hunter. 2003. Association of NAT2 and smoking in relation to breast cancer incidence in a population-based case-control study (united states). *Cancer Causes & Control* 14, (1) (Feb): 43-51.	Empirical comparison of case-only and case-control designs using real data	"Estimates are valid only if the factors are independent in the study population."		"The increased odds associated with slow acetylation were not statistically significant in the case-control analysis. In addition, there was no consistent evidence in a case-only analysis of multiplicative interaction between NAT2 and smoking."	
García-Closas, M., N. Malats, D. Silverman, M. Dosemeci,	Empirical comparison of	"[The case-only design] is a powerful design to test for		"An updated case-only meta-analysis of	"A case-only design ... removed possible

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
M. Kogevinas, D. W. Hein, A. Tardon, et al. 2005. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: Results from the spanish bladder cancer study and meta-analyses. Lancet 366, (9486) (Aug 20-26): 649-59.	case-only and case-control designs using real data; Empirical comparison of case-only meta-analysis and case-control meta-analysis using real data	multiplicative interactions under the assumption of independence of NAT2 and GSTM1 from smoking status in the population."		studies... confirmed the absence of a multiplicative interaction [as in the case-control meta-analysis]."	biases resulting from the inclusion of hospital controls with diseases related to tobacco use"
Gatto, N. M., U. B. Campbell, A. G. Rundle, and H. Ahsan. 2004. Further development of the case-only design for assessing gene-environment interaction: Evaluation of and adjustment for bias. International Journal of Epidemiology 33, (5) (Oct): 1014-24.	Theoretical evaluation	"The validity of the case-only study hinges on one assumption – that the genetic and environmental factors of interest are independent of one another." "Even when the disease risk is relatively low, controls may not provide a good approximation of the gene-environment association in the underlying population." "Using controls to approximate the G-E OR in the underlying population can lead to the rejection of valid case-only data, an overestimation of the underlying interaction, or a finding of interaction when none exists."	"When gene-gene interaction is of interest, linkage disequilibrium may cause non-independence between the genes. In this circumstance, the source of non-independence may not easily be attributable to a third variable, making adjustment impossible."	"Independence between G and E in the source population is required for equivalence between the case-only OR and the GxE relative risk."	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		"Even when disease risk is low, the G-E OR in the controls may not accurately reflect the G-E OR in the source population, Therefore, the observation of independence or non-independence between G and E among controls does not provide a consistent test for bias in case-only analysis" "The circumstances in which ORs and RRs yield materially different estimates of interaction are the same circumstances in which controls cannot be used to estimate the G-E OR in the population." "Although there are some situations in which the assessment of G-E independence in the controls will be a good proxy for assessment in the underlying population, recognizing these situations in practice requires assurance of many unknown quantities (e.g. the risk of disease among those without the genetic or the environmental factor, the			

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		underlying magnitude of interaction, etc.). Even when it is possible to estimate these quantities, minimal errors at critical thresholds can cause a researcher to come to the incorrect conclusion about whether controls may be safely used to evaluate independence." "Gene-dependent behavior modification may occur when an individual knows or can guess at his/her gene status, or when the gene status is symptomatic." "There may be alternatives for assessing independence [in controls]... for example, in a small random sample of the general population." "In case-only studies of interaction, researchers should be concerned with controlling for covariates that cause non-independence between G and E to ensure that any G-E association among cases reflects their interaction in causing disease" "The extent of bias is related			

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		to the strength and direction of the G-E association. Adjustment for C [C represents the variable(s) that cause a G-E association] in the case-only analysis (by including C in the logistic model) completely removes this bias." "We found that as long as the baseline risk of disease is small (<1%) and the interaction and independent effects are moderate (<2), controls provide a reasonable approximation of the gene-environment association in the population. Alternatively controls can be used if the disease risk is low (e.g. <5%) in all strata of G and E… however… the approximation becomes progressively worse with minimal increases in the baseline risk of disease, interaction, or independent effects." "Control for non-independence requires that the source(s) of non-independence can be conceptualized and measured.			

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	**Gene-gene interaction**		
		However, this may be difficult or impossible in some situations."			
Gauderman, J., and Millstein, J. 2002.	Theoretical evaluation				"The standard case-only approach estimates the odds-ratio between G and E in a sample of cases, without regard for T [age of onset or censoring age]. However this OR estimates G-E interaction on a relative-risk scale, and will be a biased estimator of [the interaction effect] unless D is rare or followup time is short." "Under [a G-E interaction of 2], our modified case-only analysis yielded an unbiased estimate (average estimate=2.03) while the standard case-only estimates were severely biased (average=-0.12).

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
Hamajima, N., H. Yuasa, K. Matsuo, and Y. Kurobe. 1999. Detection of gene-environment interaction by case-only studies. *Japanese Journal of Clinical Oncology* 29, (10) (Oct): 490-3.	Theoretical evaluation; Empirical comparison of case-only and case-control designs using real and simulated {{1508 Mukherjee,B. 2008; }}data	"A high prevalence of exposure and genotype in the population was more likely to demonstrate significant interaction. In such combinations, the analysis based on the case-only study was found to be fruitful, although it did not provide ORs for exposure or genotype alone." "A case-only study provides valid estimates only on the assumption that genotype and exposure are independently distributed in the study population. If they are associated, the odds ratio for interaction derived from a case-only study would be biased depending on the strength of the association." "The association [between genotype and exposure] seems to be the exception rather than the rule. In four studies used as examples in this paper, no significant association was observed between the polymorphism and smoking habit."		"[Using simulated data], analysis with the case-only study gave the same OR [as with the case-control study], but significance was achieved for some of the datasets." "[Using real data examples], there were no substantial differences in the point estimates for interaction between [case-control and case-only studies] and their 95% confidence intervals overlapped. "	
Hartge, P., N. Chatterjee, S.	Empirical			"In this study, the	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
Wacholder, L. C. Brody, M. A. Tucker, and J. P. Struewing. 2002. Breast cancer risk in ashkenazi BRCA1/2 mutation carriers: Effects of reproductive history. Epidemiology 13, (3) (May): 255-61.	comparison of case-only and kin-cohort designs using real data			case-case analysis provides less information that the kin-cohort analysis, but it uses independent data to assess interaction. Its results support the findings given by the kin-cohort approach."	
Joshi AD, Corral R, Siegmund KD, et al (2009) Red meat and poultry intake, polymorphisms in the nucleotide excision repair and mismatch repair pathways and colorectal cancer risk. Carcinogenesis 30: 472-479.	Empirical comparison of case-only and case-control designs using real data			"Our analyses using sibships [as controls] generally supported the findings [from the case-only study]."	
Keijzer, M. B., G. F. Borm, H. J. Blom, G. M. Bos, F. R. Rosendaal, and M. den Heijer. 2007. No interaction between factor V leiden and hyperhomocysteinemia or MTHFR 677TT genotype in venous thrombosis- Results of a meta-analysis of published studies and a large case-only study. Thrombosis & Haemostasis 97, (1) (Jan): 32-7.	Empirical comparison of case-only study and case-control meta-analysis using real data	"A case-only design relies on the assumption that the exposure and genotype in question are independent of another. Violation of this assumption may result in distorted study outcomes."		"Both the meta-analysis on homocysteine and factor V Leiden and …the case-only study do not show interaction in the risk for venous thrombosis."	
Khoury, M. J., and W. D.	Theoretical	"There are some genes that		"The COR obtained	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
Flanders. 1996. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: Case-control studies with no controls! American Journal of Epidemiology 144, (3) (Aug 1): 207-13.	evaluation; Empirical comparison of case-only and case-control designs using real data	may lead to a higher or lower likelihood of the exposure on the basis of some biologic mechanisms."		from [the case-only analysis] is 5.1, comparable with the [case-control odds ratio] of 6.1 obtained from the regular case-control analysis."	
Lazo-Langner, A., G. A. Knoll, P. S. Wells, N. Carson, and M. A. Rodger. 2006. The risk of dialysis access thrombosis is related to the transforming growth factor-beta1 production haplotype and is modified by polymorphisms in the plasminogen activator inhibitor-type 1 gene. Blood 108, (13) (Dec 15): 4052-8.	Empirical comparison of case-only and case-control designs using real data			"Using a 2x4 table analysis for case-control studies we determined the synergy index and using a case-only approach we determined the case-only cross-product. Both showed values greater than 1, indicating a departure from multiplicativity of effects."	
Li D, Conti DV (2009) Detecting gene-environment interactions using a combined case-only and case-control approach. Am J Epidemiol 169: 497-504.	Theoretical evaluation; Empirical comparison of case-only and case-control designs using real and simulated data	"Some authors have suggested first testing the GxE association in the controls and then choosing the analysis strategy based on the GxE test results. Unfortunately, this approach will be very dependent upon the power for declaring significance from a test		"With no GxE association, the mean squared error (MSE) is smallest for the case-only approach and largest for the case-control approach." "As the GxE association increases, the MSE and bias of	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		within the controls." "An alternative approach is to adjust for a covariate believed to lead to the GxE association in the case-only analysis. While applicable in some situations, this approach relies heavily on the presence and identification of the intermediate covariate. Such prior identification of appropriate covariates may be difficult or even impossible in practice."		the case-only approach increases sharply, while the MSE of the Bayes Model Averaging approach remains close to that of the case-control approach." "In the analysis of an SNP located on a different chromosome with the VNTR, there is an observed consistency in the estimates from the case-only and case-control analyses." "In the analysis in which there exists correlation between SNP2 and the VNTR, there is a discrepancy between the case-only and case-control estimates."	
Liu, X., M. D. Fallin, and W. H. Kao. 2004. Genetic dissection methods: Designs used for tests of gene-environment interaction. Current Opinion in Genetics & Development 14, (3) (Jun):	Theoretical evaluation; Empirical comparison of case-only and case-control designs using	"To investigate the frequency of [the G-E independence] violation empirically, we examined various GxE correlations among controls in four large genetic studies of various complex diseases		"To our surprise, we also observed that five of the seven significant interactions detected in the complete case-control sets were not detected	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
241-5.	real data	from the Johns Hopkins University. Very few GxE correlations that we could examine were statistically significant among controls, indicating that the partial-collection designs [including the case-only design] may be preferable for most investigations of GxE interaction among these studies." "Assessment of GxE correlation in the population for partial-collection designs should not be based solely on statistical significance." "When genetic effects are different in subpopulations while exposure prevalence is also different between them, G and E correlation will be induced among cases, and a case-only design can be biased."		in a case-only analysis. This argues for reduced power of the case-only approach. Actually, this was due to correlations between G and E that were in the opposite direction of the risk interaction effect. Although these correlations were not statistically significant, they diluted the ability to detect interaction in the case-only design." "OR estimated in the case-only analysis is closer to the null than the OR estimated in the complete case-control analysis because the non-statistically significant GxE correlation among controls is in the opposite direction of the interaction effect in case-control sets."	
Mukherjee B, Chatterjee N	Theoretical	"The performance of the			

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
(2008) Exploiting gene-environment independence for analysis of case-control studies: an empirical Bayes-type shrinkage estimator to trade-off between bias and efficiency. Biometrics 64: 685-694.	evaluation; Empirical comparison of case-only and case-control designs using simulated data	case-only estimator deteriorates sharply [with respect to mean squared error and bias] as one moves away from the independence assumption."			
Mukherjee B, Ahn J, Gruber SB, Rennert G, Moreno V, Chatterjee N (2008) Tests for gene-environment interaction from case-control data: a novel study of type I error, power and designs. Genet Epidemiol 32: 615-626.	Theoretical evaluation; Empirical comparison of case-only and case-control designs using real and simulated data	"To resolve the bias vs. efficiency dilemma, practitioners may naturally adapt to a two-stage procedure where, at first, one formally tests for the adequacy of the gene-environment independence assumption based on the data itself and then uses the outcome of that test to decide whether to use the powerful case-only or the more robust case-control test for estimation. For a given study of modest sample size however, the power of the tests for GxE independence would typically be low and consequently the two-stage procedure, as a whole, could still remain significantly biased. Moreover, a proper variance calculation for the		"The various association and interaction scenarios considered in the above real data example serve as an illustration of how inference could drastically change by assuming gene-environment independence when it is not true and how the conclusions from case-control and case-only analysis could be widely different depending on the G-E association present in a particular data situation."	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		two-stage estimator accounting for the underlying model uncertainty can be fairly complicated."			
Piegorsch, W. W., C. R. Weinberg, and J. A. Taylor. 1994. Non-hierarchical logistic models and case-only designs for assessing susceptibility in population-based case-control studies. Statistics in Medicine 13, (2) (Jan 30): 153-62.	Theoretical evaluation	"The case-only analysis relies on the assumption that the exposure and the genetic factor under study occur independently in the population (and that the disease is rare). This seemingly weak assumption may be violated in practice, for example, if exposure patterns and the frequency of the allele both vary with, say, ethnic group. Stratification may help achieve near-independence within the strata, but still it is hard to imagine a scenario where the control group could comfortably be dispensed with altogether."		"Based on cases alone, we get 2.8 (0.9, 8.8) and 2.3 (0.8, 7.3) for the estimated interaction parameters (and 95% CI) between the exposure and the AB and BB genetic categories, respectively. Based on the entire data, if we fit a saturated model, the corresponding estimates would be 3.3 (0.8, 13.6) and 2.5 (0.6, 10.2). Collapsing over the two genetic types we get 2.5 (1.0, 6.8) for cases only, as compared with 2.9 (0.9, 9.5) for the entire data under a saturated model."	
Schmidt, S., and D. J. Schaid. 1999. Potential misinterpretation of the case-only study to assess gene-	Theoretical evaluation	"The assumption of independence may be violated in practice if, for example, both exposure		"If genetic and environmental risk factors are independent in the	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
environment interaction. American Journal of Epidemiology 150, (8) (Oct 15): 878-85.		pattern and allele frequency vary with confounding factors such as age or ethnic group."		population, the cross-product ratio is equal to a risk ratio. If, additionally, the risk of disease is small at all levels of the study variables, it is approximately equal to the odds ratio for gene-environment interaction that is computed from case-control data." "Using only cases to assess gene-environment interaction can lead to under-estimation of the interaction odds ratio computed from case-control data because interaction is measured as departure from multiplicative risk ratios." "The cross-product ratio computed from case-only data may underestimate the population odds ratio if controls are selected from the survivors at	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
				the end of the study time (cumulative sampling), or it may under-estimate the population risk ratio if controls are selected longitudinally throughout the course of the study (density sampling). If controls are selected from the base population at the beginning of a study, both the case-control interaction odds ratio and the case-only cross product ratio estimate the interaction risk ratio. However this method of control selection is not typically used in actual case-control studies." "The interaction parameters estimated from case-only and case-control data are of comparable magnitude if the disease is truly rare (on the order of 1	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
				percent) or if the disease is somewhat more common (around 5 percent) but the genes under study confer only moderately increased disease risk (e.g., OR(G) < 6)." "For diseases as common as breast or colorectal cancer, a case-only analysis is likely to underestimate population odds or rate ratios for multiplicative interactions greater than two, but always yields unbiased estimates of interaction risk ratios. These considerations are based on the assumption that the genetic factor is associated with an increased disease risk in the absence of the environmental factor and vice versa." "The rare disease	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
				assumption is relevant only when the investigator wants to compare the actually estimated effect measure with one of the other effect measures: If the disease is rare for all exposure patterns under study during the interval of interest, the population odds ratio estimated from case-control data with cumulative sampling is approximately equal to that estimated from density-sampled case-control data and is also approximately equal to the population risk ratio estimated from case-only data under the independence assumption."	
Stucker, I., P. Boffetta, S. Antilla, S. Benhamou, A. Hirvonen, S. London, and E. Taioli. 2001. Lack of interaction between asbestos exposure and glutathione S-	Empirical comparison of pooled case-only and pooled {{1347 Tan,Q. 2007; }}case-			"[The case-only] approach, however, also failed to show any interaction between asbestos and GST polymorphism	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
transferase M1 and T1 genotypes in lung carcinogenesis. Cancer Epidemiology, Biomarkers & Prevention 10, (12) (Dec): 1253-8.	control designs using real data			[compared to the case-control analysis]."	
Tan, Q., A. I. Yashin, E. M. Bladbjerg, M. P. de Maat, K. Andersen-Ranberg, B. Jeune, K. Christensen, and J. W. Vaupel. 2002. A case-only approach for assessing gene by sex interaction in human longevity. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 57, (4) (Apr): B129-33.	Theoretical evaluation; Empirical comparison of case-only and case-control designs using real data		"First, in order to apply this method, researchers must assume that sex and the genotype are independent." "[The independence] assumption holds for any autosomal genes because their segregation does not depend on sex. However, one must note that for sex-linked genes, such an application definitely violates the basic assumption." "The event associated with gene by sex interaction should be rare." "Although in the context of gene by sex interaction, such an assumption holds	"The results [of the case-control and case-only analysis of CVD-associated genes] are compatible." "The case-only odds ratio…is compatible with the conclusion drawn from the case-control approach."	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
			provided the genes of interest are autosomal, caution has to be paid when we want to apply the same approach to study GXE interactions. In the latter situation, especially when the environment relates to geographical allocation, it is important to check the ethnic origin of the populations to make sure that the assumption is satisfied."		
Theodoratou, E., H. Campbell, A. Tenesa, G. McNeill, R. Cetnarskyj, R. A. Barnetson, M. E. Porteous, M. G. Dunlop, and S. M. Farrington. 2008. Modification of the associations between lifestyle, dietary factors and colorectal cancer risk by APC variants. Carcinogenesis (Mar 28).	Empirical comparison of case-only and case-control designs using real data			"There was statistical evidence to suggest that there is an interaction effect greater than the one expected from a multiplicative model (case-control design). In addition, according to the case-only analysis APC 1822 interacted significantly with HRT." "Results from interaction analyses (case-control and case-	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
				only designs) for the APC 1317 were not significant." "According to the case-only analysis, APC 1822 interacted significantly with red meat and MUFA, results which are in accordance with the case-control analysis." "Results from the interaction analyses (case-control and case-only designs) for the APC 1317 [and fat intake] were not significant."	
Vittinghoff, E., and D. C. Bauer. 2006. Case-only analysis of treatment-covariate interactions in clinical trials. Biometrics 62, (3) (Sep): 769-76.	Theoretical evaluation; Empirical comparison of case-only, Cox model, and case-cohort analyses using simulated and real data	"The case-only method would likely be reliable… when overall event rates ≤20%,… and when early drop out rates are nondifferential by treatment and subgroup, or are less than 10%."		"For the setting with uniform 10% early drop out across treatment and subgroup, [the case-only] MSE is substantially smaller than for the case-cohort analysis and only moderately greater than for the Cox model." "The results [using real data] show close	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
				quantitative and qualitative concordance between the case-only and Cox model results." "Case-only analysis gave results that were in general more consistent with the Cox model results than case-cohort analyses." "Our simulations confirmed that under a wide range of plausible circumstances, the case-only method would outperform a case-cohort approach for examining treatment-covariate interaction."	
Wang L-, Lee W- (2008) Population stratification bias in the case-only study for gene-environment interactions. Am J Epidemiol 168: 197-201.	Theoretical evaluation	"It is clear from the formula above for the confounding interaction ratio (CIR) that there would be no population stratification bias if, across the strata, 1) the exposure prevalence odds and the genotype frequency odds are uncorrelated, 2) there is no variation in the exposure			

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		prevalence odds, or 3) there is no variation in the genotype frequency odds. Also, one notices that overestimation (CIR>1) would occur when the prevalence odds of exposure and the frequency odds of genotype are positively correlated, and underestimation (CIR<1) would occur when these are negatively correlated. Higher relative bias (CIR away from 1) exists with higher variation of the prevalence odds of exposure or the frequency odds of exposure." "The estimated interaction effect in a case-only study is frequently biased by more than 5 percent, a nonnegligible amount of bias. For a rarer gene and/or a rarer exposure, the bias becomes larger." "We have shown that, in addition to the bias due to gene-environment nonindependence, the bias due to population stratification is also a major			

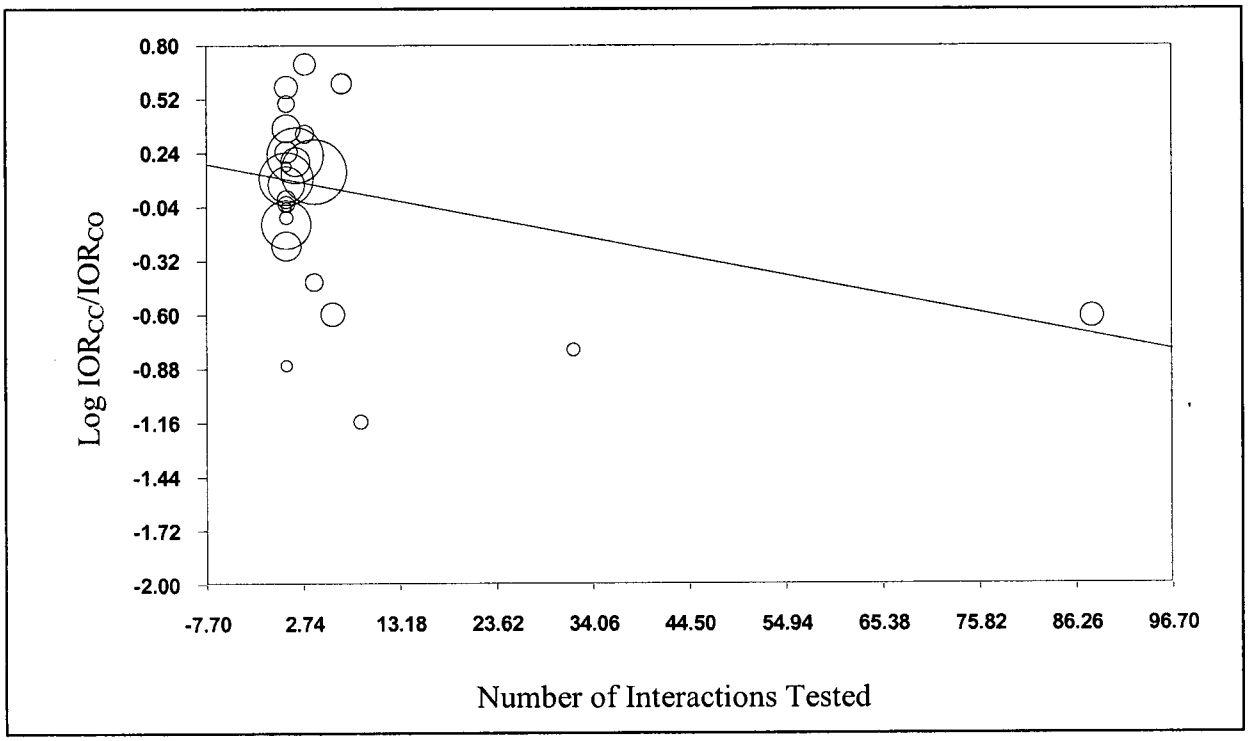
Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
		threat to the validity of a case-only study."			
Yang, Q., M. J. Khoury, F. Sun, and W. D. Flanders. 1999. Case-only design to measure gene-gene interaction. Epidemiology 10, (2) (Mar): 167-70.	Theoretical evaluation; Empirical comparison of case-only and case-control designs using real data		"Under the assumption of independent gene frequencies in the population, the case-only cross-product provides an estimate of the ratio of the joint effect divided by the product of the individual effects of each gene alone, which can be regarded as effect measure modification of risk ratio on a multiplicative scale or a gene-gene interaction of the risk ratio." "For a rare disease, risk ratios approximate odds ratios so that our results imply that the cross-product measures depart from a multiplicative relation	"Using the case-only design, we estimate an interaction risk ratio of 2.0 (95% CI 0.6-6.0) for gene-gene interaction, also implying interaction."	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
			of odds ratios. Our results, however, also show that the cross-product remains a valid measure of departure from multiplicativity of risk ratios even if the disease is not rare." "The probability of correlations (linkage disequilibrium) between genes on the same chromosome will increase, especially for those that are physically close to eachother." "The effects of population stratification may also invalidate the results of a case-only study."		
Yang, C. X., K. Matsuo, H. Ito, M. Shinoda, S. Hatooka, K. Hirose, K. Wakai, et al. 2005. Gene-environment interactions between alcohol drinking and the MTHFR C677T polymorphism impact on esophageal cancer risk: Results of a case-control study in Japan. Carcinogenesis 26, (7)	Empirical comparison of case-only and case-control designs using real data			"The significant finding in this study was the interaction between MTHFR TT genotype and heavy alcohol drinking with both case-only and case-control designs."	

Citation [Author(s), Publication Date, Title, Journal]	Study Type	Sources and extent of bias in the case-only design applied to studies of:		Comparison of case-only design to other study designs (e.g. case control, cohort, etc)	Other comments related to case-only design
		Gene-environment interaction	Gene-gene interaction		
(Jul): 1285-90.					
Zeiger, J. S., T. H. Beaty, and K. Y. Liang. 2005. Oral clefts, maternal smoking, and TGFA: A meta-analysis of gene-environment interaction. Cleft Palate-Craniofacial Journal 42, (1) (Jan): 58-63.	Empirical comparison of case-only meta-analysis and case-control meta-analysis using real data			"The joint effect of maternal smoking and infant genotype [in determining cleft palate] reached marginal statistical significance in the polytomous logistic regression … Case-only analysis revealed significant gene-smoking interaction under a multiplicative model." "The joint effect of smoking and genotype [in determining cleft lip ± cleft palate] was not statistically significant, however. Case-only analysis also showed no evidence of gene-environment interaction."	

Appendix D

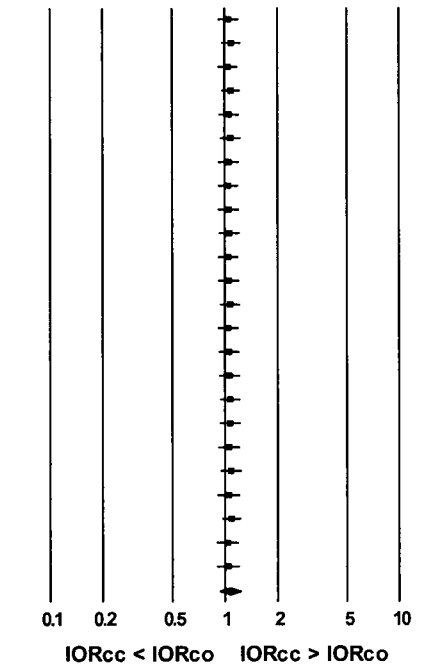
Univariable regression of the number of interactions tested on the log IOR_{CC}/IOR_{CO} ratio. The size of the circles reflects the weight of the study in the meta-analysis.



Appendix E

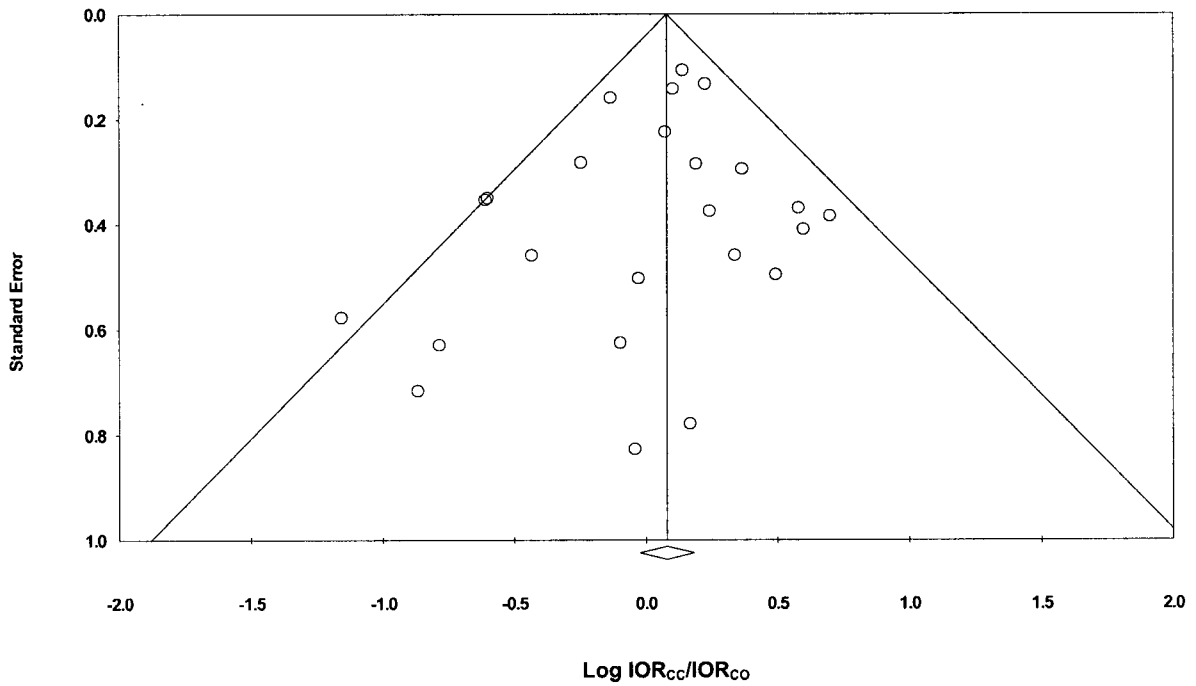
Mean IOR_{CC}/IOR_{CO} ratio (95% CI) with removal of one study at a time under a random effect model.

Study name	Statistics with study removed				
	Point	Lower limit	Upper limit	Z-Value	p-Value
Khoury, 1996	1.053	0.913	1.213	0.706	0.480
Yang, 2005	1.089	0.958	1.238	1.300	0.194
Caceres, 2009	1.045	0.915	1.194	0.647	0.518
Caceres, 2005	1.084	0.958	1.227	1.277	0.202
Lazo-Langer, 2006	1.060	0.921	1.219	0.807	0.420
Albert, 2001	1.077	0.936	1.238	1.037	0.300
Yang, 1999	1.051	0.915	1.208	0.703	0.482
Hamajima, 1999 (Wu, 1997)	1.045	0.912	1.197	0.634	0.526
Stücker, 2001 (London, 1995)	1.051	0.910	1.214	0.681	0.496
Stücker, 2001 (Jourenkova, 1997)	1.061	0.922	1.221	0.822	0.411
Egan, 2003	1.047	0.913	1.200	0.657	0.511
Dowling, 2003	1.053	0.914	1.212	0.714	0.475
Clavel, 2005	1.072	0.937	1.227	1.018	0.309
Hamajima, 1999 (Taylor, 1998)	1.045	0.908	1.203	0.610	0.542
Hamajima, 1999 (Sugimura, 1998)	1.058	0.919	1.217	0.783	0.433
Stücker, 2001 (Stücker, 1999)	1.060	0.921	1.221	0.812	0.417
Hamajima, 1999 (Hildesheim, 1997)	1.071	0.936	1.225	0.993	0.321
Deng, 2004	1.072	0.934	1.230	0.989	0.323
Li, 2009	1.056	0.912	1.223	0.733	0.463
Chang-Claude, 2003	1.084	0.939	1.251	1.098	0.272
Zeiger, 2005	1.060	0.902	1.223	0.629	0.529
Theodoratou, 2008	1.089	0.958	1.238	1.299	0.194
Garcia-Closas, 2005	1.035	0.892	1.201	0.457	0.648
Mukherjee, 2008	1.042	0.892	1.218	0.523	0.601
	1.061	0.925	1.216	0.844	0.399



Appendix F

Funnel plot of studies included in meta-regression analysis.



Appendix G

Interaction between total alcohol consumption in drinks/week (dichotomized at the median of all cases) and *BRCA1* and *BRCA2* gene mutations stratified by potential confounding factors.

Potential Confounding Factor	***BRCA1*** **IOR_{CO} (95% CI) (unadjusted)**	***BRCA2*** **IOR_{CO} (95% CI) (unadjusted)**
Time between diagnosis and interview		
<3 years	1.07 (0.21-5.33)	1.87 (0.77-4.53)
≥3 years	0.36 (0.04-3.57)	1.93 (0.56-6.72)
BMI		
<25	0.76 (0.20-2.86)	1.24 (0.53-2.87)
≥25	-	5.72 (1.19-27.47)
Age at diagnosis		
<50 years	0.64 (0.15-2.71)	1.49 (0.65-3.43)
≥50 years	1.11 (0.07-17.88)	3.88 (0.79-18.98)
Menopause		
Premenopausal	0.80 (0.18-3.61)	1.33 (0.51-3.44)
Postmenopausal	0.56 (0.05-6.20)	3.07 (0.96-9.79)
Oral Contraceptive Use		
Never	-	-
Ever	0.63 (0.15-2.63)	1.82 (0.88-3.76)
HRT Use		
Never	0.65 (0.15-2.75)	1.84 (0.83-4.09)
Ever	1.08 (0.07-17.44)	2.15 (0.39-12.02)
Parity		
0	1.30 (0.21-7.89)	0.87 (0.12-6.23)
>0	0.30 (0.03-2.73)	2.31 (1.05-5.05)
Smoking		
Never	0.51 (0.05-4.97)	0.92 (0.22-3.92)
Ever	0.89 (0.18-4.43)	2.28 (0.94-5.55)

- Model fit questionable

HRT=Hormone replacement therapy; BMI=Body mass index

Appendix H

Interaction between alcohol consumption in drinks/week (dichotomized at the median of all cases who consumed alcohol) and *BRCA1* and *BRCA2* gene mutations among cases who consumed alcohol in the year prior to diagnosis.

Model	≤median (No. non-carriers / No. carriers)	>median (No. non-carriers / No. carriers)	p-value[a]	IOR_{CO} (95% CI) (unadjusted)	IOR_{CO} (95% CI) (adjusted)[b]
BRCA1					
Total Alcohol (median=4 drinks/week)	349/7	338/3	0.34	0.44 (0.11-1.73)	0.49 (0.13-1.94)
Wine (median=2 drinks/week)	351/8	336/2	0.11	0.26 (0.06-1.24)	0.28 (0.06-1.35)
Other Alcohol[c] (median=0.5 drinks/week)	364/4	323/6	0.53	1.69 (0.47-6.04)	1.80 (0.50-6.48)
BRCA2					
Total Alcohol (median=4 drinks/week)	349/11	338/19	0.13	1.78 (0.84-3.80)	1.92 (0.89-4.11)
Wine (median=2 drinks/week)	351/13	336/17	0.41	1.37 (0.65-2.86)	1.44 (0.69-3.03)
Other Alcohol[c] (median=0.5 drinks/week)	364/11	323/19	0.08	1.95 (0.91-4.15)	2.04 (0.95-4.37)

[a]Based on Chi-squared or Fisher's exact test

b Adjusted for age at diagnosis

[c]Includes beer, fortified wine, and spirits

Appendix I

Interaction between alcohol consumption in drinks/week (dichotomized at the median of all cases) and *BRCA2* gene mutations among cases, adjusted for age at diagnosis and family history of breast/ovarian cancer. All *BRCA1* mutation carriers had a family history of breast/ovarian cancer, resulting in quasi-complete separation of the model. Family history was defined as two or more first or second degree relatives with breast and/or ovarian cancer.

Model	**≤median (No. non-mutation carriers / No. mutation carriers)**	**>median (No. non-mutation carriers / No. mutation carriers)**	**p-value[a]**	**IOR_{CO} (95% CI) (unadjusted)**	**IOR_{CO} (95% CI) (adjusted)[b]**
BRCA2					
Total Alcohol (median=3 drinks/week)	423/12	391/21	0.08	1.89 (0.92-3.90)	1.94 (0.94-4.03)
Wine (median=2 drinks/week)	478/16	336/17	0.24	1.51 (0.75-3.03)	1.53 (0.76-3.09)
Other Alcohol[c] (median=0.33 drinks/week)	420/11	394/22	0.04	2.13 (1.02-4.45)	2.22 (1.06-4.67)

[a]Based on Chi-squared or Fisher's exact test

b Adjusted for age at diagnosis and family history of breast/ovarian cancer

[c]Includes beer, fortified wine, and spirits

Appendix J

Association between breast cancer risk and the number of alcoholic drinks consumed per week among *BRCA1* and *BRCA2* mutation carrier pairs in which both the case and control consumed alcohol.

Model	Number of Alcoholic Drinks Consumed per Week			
	0-3	4-9	≥10	p-trend
BRCA1 (n=1034 pairs)				
Cases/controls	406/407	89/79	22/31	
Univariable OR (95% CI)	1.00	1.14 (0.80-1.61)	0.73 (0.42-1.26)	0.64
Multivariable OR (95% CI)[a]	1.00	1.14 (0.78-1.67)	0.66 (0.37-1.20)	0.50
BRCA2 (n=432 pairs)				
Cases/controls	158/158	45/46	13/12	
Univariable OR (95% CI)	1.00	0.98 (0.60-1.60)	1.08 (0.43-2.40)	0.93
Multivariable OR (95% CI)[a]	1.00	1.09 (0.64-1.85)	1.46 (0.59-3.61)	0.45

[a]Adjusted for ethnicity (other white, French-Canadian, Jewish, other), menopause, oral contraceptive use, HRT use, smoking, oophorectomy, BMI (<25, 25 to <30, ≥30), and parity (0, 1, 2, 3, 4+)

HRT=Hormone replacement therapy; BMI=Body mass index

Appendix K

Association between breast cancer risk and the number of alcoholic drinks consumed per week among *BRCA1* and *BRCA2* mutation carriers by age at diagnosis, BMI, and smoking. The referent group is non-drinkers (675 *BRCA1* cases, 612 *BRCA1* controls; 148 *BRCA2* cases, 141 *BRCA2* controls).

Model	Number of Alcoholic Drinks Consumed per Week			
	0-3 Multivariable OR (95% CI)[a]	4-9 Multivariable OR (95% CI)[a]	≥10 Multivariable OR (95% CI)[a]	p-trend
BRCA1 (n=1480 pairs)				
Age at Diagnosis				
<50	0.84 (0.70 to 1.00)	1.04 (0.76 to 1.43)	0.56 (0.34 to 0.95)	0.10
≥50	0.52 (0.29 to 0.95)	0.24 (0.06 to 0.95)	0.35 (0.04 to 3.16)	0.01
BMI				
<25	0.76 (0.62 to 0.93)	0.81 (0.54 to 1.22)	0.41 (0.20 to 0.80)	0.004
≥25	0.77 (0.59 to 1.01)	0.55 (0.28 to 1.11)	0.69 (0.18 to 2.71)	0.03
Unknown	0.83 (0.63 to 1.09)	1.50 (0.93 to 2.41)	0.71 (0.31 to 1.62)	0.92
Smoking				
No	0.76 (0.63 to 0.92)	0.97 (0.65 to 1.47)	0.31 (0.13 to 0.76)	0.01
Yes	0.87 (0.71 to 1.06)	1.04 (0.72 to 1.52)	0.78 (0.43 to 1.43)	0.37
BRCA2 (n=445 pairs)				
Age at Diagnosis				
<50	0.91 (0.60 to 1.37)	0.95 (0.58 to 1.55)	1.16 (0.50 to 2.69)	0.96
≥50	1.41 (0.45 to 4.40)	1.54 (0.33 to 7.16)	0.84 (0.09 to 7.55)	0.78
BMI				
<25	1.01 (0.66 to 1.54)	1.02 (0.60 to 1.74)	0.85 (0.34 to 2.12)	0.72
≥25	0.58 (0.33 to 1.02)	1.29 (0.53 to 3.15)	3.68 (0.31 to 43.51)	0.61
Unknown	1.08 (0.61 to 1.89)	0.80 (0.32to 1.98)	1.63 (0.40 to 6.58)	0.66
Smoking				
No	1.09 (0.72 to 1.63)	1.05 (0.56 to 1.96)	0.67 (0.17 to 2.74)	0.64
Yes	0.76 (0.48 to 1.21)	0.95 (0.55 to 1.64)	1.20 (0.52 to 2.81)	0.79

[a]Adjusted for ethnicity (other white, French-Canadian, Jewish, other), menopause, oral contraceptive use, HRT use, smoking, oophorectomy, BMI (<25, 25 to <30, ≥30), and parity (0, 1, 2, 3, 4+)

HRT=Hormone replacement therapy; BMI=Body mass index

Appendix L

Association between breast cancer risk and the number of alcoholic drinks consumed per week among *BRCA1* and *BRCA2* mutation carriers interviewed within 5 years of diagnosis.

Model	Number of Alcoholic Drinks Consumed per Week				
	None	0-3	4-9	≥10	p-trend
BRCA1 (n=794 pairs)					
Multivariable OR (95% CI)[a]	1.00	0.70 (0.55-0.89)	1.05 (0.68-1.60)	0.45 (0.21-0.95)	0.04
BRCA2 (n=247 pairs)					
Multivariable OR (95% CI)[a]	1.00	0.90 (0.54-1.50)	0.95 (0.50-1.81)	0.40 (0.12-1.34)	0.32

[a]Adjusted for ethnicity (other white, French-Canadian, Jewish, other), menopause, oral contraceptive use, HRT use, smoking, oophorectomy, BMI (<25, 25 to <30, ≥30), and parity (0, 1, 2, 3, 4+)

HRT=Hormone replacement therapy; BMI=Body mass index

Appendix M

Association between breast cancer risk and current alcohol consumption by the type(s) of alcohol typically consumed among *BRCA1* and *BRCA2* mutation carriers interviewed within 5 years of diagnosis. The referent group is non-drinkers.

Model	Multivariable OR (95% CI)[a]
BRCA1 (n=457 pairs)	
Exclusive wine consumers	0.48 (0.30-0.76)
Wine and other alcohol types[b]	0.94 (0.62-1.42)
Other alcohol types[b]	0.70 (0.41-1.22)
BRCA2 (n=125 pairs)	
Exclusive wine consumers	0.90 (0.37-2.17)
Wine and other alcohol types[b]	0.42 (0.17-1.02)
Other alcohol types[b]	0.86 (0.25-3.00)

[a]Adjusted for ethnicity (other white, French-Canadian, Jewish, other), menopause, oral contraceptive use, HRT use, smoking, oophorectomy, BMI (<25, 25 to <30, ≥30), and parity (0, 1, 2, 3, 4+)

[b]Includes beer and spirits

HRT=Hormone replacement therapy; BMI=Body mass index